AMITY LIPIDOMICS RESEARCH FACILITY (ALRF)

AMITY UNIVERSITY GURUGRAM

AMITY LIPIDOMICS RESEARCH FACILITY (ALRF)

INTRODUCTION

Amity Lipidomics Research Facility (ALRF) was established at the Amity University, Haryana in 2018, with funding from Department of Science and Technology (DST-FIST) Government of India and Amity University, Haryana. The facility was made available for research and commercial purposes from September 2019.

The purpose of establishing this facility is to provide the scientific community (within Amity University and external researchers, both academia and industry) with state-of-the-art mass spectrometric techniques for separation, identification, and quantitation of biomolecules like lipids, metabolites, and proteins.

Amity Lipidomics Research Facility houses a triple stage quadrupole/linear ion trap mass spectrometer, Q-Trap 4500 (SCIEX), equipped with ion sources (turbo V) Electrospray ionization (ESI), and Atmospheric pressure chemical ionization (APCI), auto sampler, column oven with HPLC (Exion LC-AC) system. The system is ideal for laboratories requiring ultimate robustness, ruggedness, and reliability for high-through put screening of many compounds in multiple samples on a daily basis.

AMITY LIPIDOMICS RESEARCH FACILITY (ALRF)

With the quantitative performance of a triple quad system and additional enhanced scan functionality of QTRAP, we are developing new methods and at the same time improving results for our existing workflow. The integrated linear ion trap (LIT) enables more accurate detection, quantification, and confirmation of compounds – without additional laborious or time-consuming sample preparation. The enhanced product ion (EPI) functionality of QTRAP allows acquisition of a complete MS/MS spectrum to accompany MRM quantitation for every compound detected in a sample. This compound's fingerprint can be cross referenced with an integrated library, which helps in delivering ultimate confirmation and reporting our analysis without doubt. The unique ability of QTRAP 4500 to capture MRM and enhanced product ion confirmation scans in one injection, without the need for long and inefficient chromatography, enables reliable screening for more compounds in our sample, without compromising data quality. This leads to better throughput, without investing more time and resources.

ALRF provides LC/MS based services to researchers and industries. The facility operates 24×7. The services on payment basis are offered and are mentioned below:

- ESI-MS based analysis (Nominal mass/ Molecular weight determination)
- MS/MS analysis
- UPLC-MS analysis (qualitative)
- UPLC-MS/MS analysis (qualitative)
- UPLC-MS/MS for targeted analysis of lipids/small molecules
- UPLC-MS/MS for targeted analysis of lipids/small molecules including data analysis by MultiQuant Software.
- UPLC-MS/MS for untargeted analysis of lipids/small molecules
- UPLC-MS/MS for untargeted analysis of lipids/small molecules including data analysis by LipidView Software.

ALRF AT A GLANCE

New Funded Projects (Total Resource Mobilization)	**Rs. ~6 Crore**
Equipment Acquired Under New Funded Projects (Total New Assets Created)	**Rs.70,46,194.00**
Revenue Generated (User Charges, Symposiums, Workshops)	**Rs.3,20,684.00**
Total Manpower Trained	**15**
Total Ph. D.'s Produced	**9**
Total Ongoing Ph. D.'s	**12**
Total Publications	**19**
Total Patents	**1**
Total Workshops/ Symposiums Conducted	**4**

AMITY LIPIDOMICS RESEARCH FACILITY (ALRF)

FOCUSED RESEARCH GROUPS

PI: Dr Ujjaini Dasgupta

Sphingolipid biology

Research in the lab focuses on decoding the intricacies of sphingolipid signalling in tumour progression using multidisciplinary approaches including lipidomics, as a major tool. In collaboration with clinicians the group works with human tumour tissue samples with the objective to identify sphingolipid-based biomarkers that has therapeutic potential. The areas of research include studying epigenetic, transcriptional and post-transcriptional mechanisms regulating diverse signalling pathways targeting the tumour microenvironment in response to nanomedicine. All projects in the lab have mass spectrometry component and hence depends primarily on the DST-FIST ALRF.

Researchers:

Kajal Rajput

Pankaj Sharma

Trishna Pani

Devashish Mehta

Md. Nafees Ansari

Nihal Medatwal

PI: Dr Zeeshan Fatima

Antimicrobial Drug Resistance

Infectious (mycobacterium and human fungal pathogens) and chronic diseases (brain stroke and diabetes); natural compounds and AYUSH drugs, micronutrient stress and lipid biology. Poor understanding of the basic biology of bacterium hampers development of much-needed drugs, vaccines, and diagnostic tests. High-throughput “omics” techniques have been applied to the study of MTB biology in recent years to address questions at the systems level. Genomic studies are increasing our understanding of MTB evolution and the development of drug resistance, while proteomics and lipidomics have enhanced our understanding of the real-time physiological status of bacilli in the host. The lab is engaged in understanding lipid biology in persistence, pathogenesis, and drug resistance of MTB.

Researchers:

Rahul Pal

Sharda Sharma

Himanshu Sharma

PI: Dr Saif Hameed

Medical Microbiology

Despite the current understanding of major factors which contribute to MDR mechanisms, there are evidences to suggest that it is a complex interplay of multiple factors which may be contributed by still unknown mechanisms. Improved knowledge of such molecular mechanisms controlling MDR in pathogenic fungi should facilitate the development of novel therapies to combat these intransigent infections. Currently, lab is inclined to work on following three strategies: Identification of natural compounds that can be used as effective antifungal agents with minimum side effects and cost effectiveness. Targeting crucial micronutrients acquisition mechanisms and how they can be exploited as efficient anti-microbial strategy or drug targets. High throughput lipidomics to evaluate unique lipid imprints that may be responsible for MDR acquisition.

Researchers:

Moiz Ashraf Ansari

Shweta Singh

Sandeep Hans

PI: Prof Rajendra Prasad

Molecular Mycology-antifungal resistance.

Work focuses on fungal biology, particularly clinical antifungal resistance. Role of membrane multidrug transporters in pathogenic yeasts and their genetic and functional characterization are some of the areas. Our earlier observations from yeasts have revealed an intricate relationship between SPH and ergosterol homeostasis vs drug susceptibility. The focus is as to how imbalances in membrane lipid homeostasis impact clinical antifungal resistance. The comprehensive high-throughput sphingolipidomics, combined with genetic approach are expected to reveal the functional interactions between SPHs, pathogenesis and MDR determinants and will also provide a detailed sphingolipidomics/lipidomics resource for the fungal lipid research community in addition to discovering a potential target for antifungal therapy. The work and these ongoing projects heavily depend on FIST-DST facility.

Researchers:

Mohit Kumar

Garima Shahi

Basharat Ali

Rajlaxmi Yadav

Praveen Kumar

Anshu

Amandeep Saini

Sonam Kumari

Mohd. Wasi

Syed Bilal Jilani

Suman Sharma

Publications Resulting out of ALRF/FISTFacilityinLipidomics area:

- Pal, S., Medatwal, N., Kumar, S., Kar, A., Komalla, V., Yavvari, P.S., Mishra, D., Rizvi, Z.A., Nandan, S., Malakar, D., Pillai, M., Awasthi, A., Das, P., Sharma, R. D., Srivastava, A., Sengupta, S., Dasgupta, U. and Bajaj, A., 2019. A Localized Chimeric Hydrogel Therapy Combats Tumor Progression through Alteration of Sphingolipid Metabolism. ACS Central Science, 5(10), pp.1648-1662.
- Hans, S., Purkait, D., Nandan, S., Bansal, M., Hameed, S. and Fatima, Z.,2020. Rec A disruption unveils cross talk between DNA repair and membrane damage, efflux pump activity, biofilm formation in Mycobacterium smegmatis. Microbial Pathogenesis, p.104262.
- Kumar, M., Singh, A.,Kumari, S., Kumar, P., Wasi, M., Mondal, A.K., Rudramurthy, S.M., Chakrabarti, A., Gaur, N.A., Gow, N.A. and Prasad, R., 2020. Sphingolipidomics of drug resistant Candida auris clinical isolates reveal distinct sphingolipid species signatures. Biochimica et BiophysicaActa (BBA)-Molecular and Cell Biology of Lipids, 1866(1), p.158815
- Shahi, G., Kumar, M., Kumari, S.,Rudramurthy, S.M., Chakrabarti, A., Gaur, N.A., Singh, A. and Prasad, R., 2020. A detailed lipidomic study of human pathogenic fungi Candida auris. FEMS Yeast Research, 20(6), p. foaa045.
- Medatwal, N., Ansari, M.N., Kumar, S., Pal, S., Jha, S.K., Verma, P., Rana, K., Dasgupta, U. and Bajaj, A., 2020. Hydrogel-mediated delivery of celastrol and doxorubicin induces a synergistic effect on tumor regression via upregulation of ceramides. Nanoscale, 12(35), pp.18463-18475.
- Prasad, R., Shukla, S., & Singh, A. (2017). Insights into candida lipids. Candida albicans: Cellular and Molecular Biology, 417-428.
- Kumar, S., Thakur, J., Yadav, K., Mitra, M., Pal, S., Ray, A., Gupta, S., Medatwal, N., Gupta, R., Mishra, D., Rani, P., Padhi, S., Sharma, P., Kapil, A., Srivastava, A., Priyakumar, U.D., Dasgupta, U.,Thukral, L. and Bajaj, A., 2019. Cholic Acid-Derived Amphiphile which Combats Gram-Positive Bacteria-Mediated Infections via Disintegration of Lipid Clusters. ACS Biomaterials Science & Engineering, 5(9), pp.4764-4775.
- Sharma, S.,Hameed, S. and Fatima, Z., 2020. Monoterpenoid Geraniol Improves Anti-Mycobacterial Drug Efficiency by Interfering with Lipidome and Virulence of Mycobacteria. Infectious Disorders-Drug Targets (Formerly Current Drug Targets-Infectious Disorders), 20(4), pp.467-485.
- Pal, R., Hameed, S. and Fatima, Z., 2019. Altered drug efflux under iron deprivation unveils abrogated MmpL3 driven mycolic acid transport and fluidity in mycobacteria. Biometals, 32(1), pp.49-63.
- Pal R, Hameed S, Kumar P, Singh S, Fatima Z. Understanding lipidomic basis of iron limitation induced chemosensitization of drug-resistant Mycobacterium tuberculosis. 3 Biotech. 2019 Apr;9(4):1-9.
- Fatima, Z. and Hameed, S., 2019. Lipidomic insight of anticandidalperillyl alcohol and sesamol induced Candida membrane disruption. Infectious Disorders Drug Targets.
- Singh, A., Khandelwal, N. K., &Prasad, R. (2019). Lipidomics Approaches: Applied to the Study of Pathogenesis in Candida Species. Yeasts in Biotechnology and Human Health, 195-215.
- Sharma, S., Hameed, S. and Fatima, Z., 2019. Lipidomic insights to understand membrane dynamics in response to vanillin in Mycobacterium smegmatis. International Microbiology, pp.1-14.
- Singh, A., Bhardwaj, N., &Prasad, R. (2020). Nanomaterial-Assisted Mass Spectrometry: An Evolving Cutting-Edge Technique. In NanoBioMedicine (pp. 453-464). Springer, Singapore.
- Kumar, A., Banerjee, A., Singh, A., &Prasad, R. (2020). Background of Membrane Lipids. In Analysis of Membrane Lipids (pp. 1-11). Springer, New York, NY.
- Medatwal, N.,&Dasgupta, U. (2020). Quantitation of Sphingolipids in Mammalian Cell Lines by Liquid Chromatography–Mass Spectrometry. In Analysis of Membrane Lipids (pp. 103-117). Springer, New York, NY.
- Pani, T., Rajput, K.,Kar, A., Sharma, H., Basak, R., Medatwal, N., Saha, S., Dev, G., Kumar, S., Gupta, S., Mukhopadyay, A., Malakar, D., Maiti, TN., Arimbasseri, AG., Deo, SVS., Sharma, RD., Bajaj, A., &Dasgupta, U. (2021). Alternative splicing of ceramide synthase 2 alters levels of specific ceramides and modulates cancer cell proliferation and migration in Luminal B breast cancer subtype. Cell death & disease, 12(2), 1-22.
- Pal, S., Soni, V., Kumar, S., Jha, SK., Medatwal, N., Rana, K., Yadav, P., Jain, D., Mehta, D., Sharma, P., Kar, r., Srivastava, A., Patil, VS., Dasgupta, U., Nandicoori, VK., and Bajaj, A. (2021). A Hydrogel-based Implantable Multidrug Antitubercular Formulation Outperforms Oral Delivery. Nanoscale, In press https://doi.org/10.1039/D0NR08806D

Patents Resulting out of ALRF/FISTFacilityinLipidomics area:

- UjjainiDasgupta, Ravi Datta Sharma, “A method providing spliced transcripts of CERS2 gene to alter the level of ceramides to effect tumor regression", ApplicationNo:202011011239.

AMITY UNIVERSITY HARYANA

HUMAN RESOURCE DEVELOPMENT
Ph.D.'s completed using ALRF Facility

Sr. No.	Name	Thesis Title	Supervisor Name	Co PI Name
1	Mohit Kumar	Sphingolipid metabolism in Candida species.	Dr Rajendra Prasad, AUH	Dr Naseem A. Gaur, ICGEB
2	Rahul Pal	Effect of iron deprivation on drug resistance of Mycobacterium tuberculosis	Dr Zeeshan Fatima, AUH	Dr Saif Hameed, AUH
3	Nihal Medatwal	Deciphering the role of sphingolipids in cancer progression and impact of chemotherapy on sphingolipid signaling	Dr Avinash Bajaj, RCB	Dr Ujjaini Dasgupta, AUH
4	Moiz Ashraf Ansari	Antifungal Effect of Natural Phenolic Compound(s) Against Human Fungal Pathogen	Dr Saif Hameed, AUH	Dr Zeeshan Fatima, AUH
5	Sharda Sharma	Antimycobacterial potential of Vanillin & Geraniol against Mycobacterium	Dr Zeeshan Fatima, AUH	Dr Saif Hameed, AUH
6	Shweta Singh	Antifungal potential of Terpenoid and Alkaloid compounds against human fungal pathogen, Candida albicans	Dr Saif Hameed, AUH	Dr Zeeshan Fatima, AUH
7	Mohd. Wasi	Identification and molecular characterisation of ABC transporters in yeasts	DrAlokMondal, JNU	Dr Rajendra Prasad, AUH
8	Sonam Kumari	Role of ABC transporters in candida species	Dr Naseem A. Gaur, ICGEB	Dr Rajendra Prasad, AUH
9	Syed Bilal Jilani	Identification of genes invovled in enhancing performance of ethanologenic e. Coli in presence of inhibitors	Dr Syed Shams Yazdani, ICGEB	Dr Rajendra Prasad, AUH

ONGOING PH.D.'S USING ALRF FACILITY

Sr. No.	Name	Thesis Title	PI Name	Co PI Name
1	Praveen Kumar	Insights into the mechanism of directed evolution of drug resistance in Candida	Dr Rajendra Prasad, AUH	-Dr Shivaprakash Rudramurthy, PGIMER, Chandigarh -Dr Atanu Banerjee, AUH
2	Anshu	Unravelling Polyene resistance mechanism(s) in the pathogen Candida auris	Dr Rajendra Prasad, AUH	-Dr Alok Mondal, JNU -Dr Atanu Banerjee, AUH
3	Rajlaxmi Yadav	Unravelling Polyene resistance mechanism(s) in the pathogen Candida auris	Dr Rajendra Prasad, AUH	Dr Naseem A. Gaur, ICGEB
4	Suman Sharma	Drug Transporters in human pathogenic yeast	Dr Rajendra Prasad, AUH	Dr Alok Mondal, JNU
5	Amandeep Saini	ABC Transporters and their role in cellular physiology and antifungal response of Candida auris	Dr Rajendra Prasad, AUH	Dr Avinash Bajaj, RCB
6	Garima Shahi	Lipids in clinical drug resistance in yeast	Dr Rajendra Prasad, AUH	Dr Naseem A. Gaur, ICGEB
7	Kajal Rajput	Comparative sphingolipid profiling of breast cancer cell and tissue types for identification of potential biomarkers.	Dr Ujjaini Dasgupta, AUH	Dr Avinash Bajaj, RCB
8	Himanshu Sharma	Understanding cell membrane dynamics of Mycobacterium species	Dr Zeeshan Fatima, AUH	Dr Saif Hameed, AUH
9	Sandeep Hans	Effect of Magnesium stress on Cellular circuitry governing drug resistance of Candida albicans	Dr Saif Hameed, AUH	Dr Zeeshan Fatima, AUH
10	Trishna Pani	Transcriptional and post transcriptional regulation of sphingolipid genes in breast cancer	Dr Ujjaini Dasgupta, AUH	-
11	Devashish Mehta	Engineering of Bile Acid-derived biomaterials for Cancer Therapy	Dr Ujjaini Dasgupta, AUH	-
12	Basharat Ali	Sphingolipidomics and drug resistance in pathogenic fungi	Dr Alok Mondal, JNU	Dr Rajendra Prasad, AUH

AMITY LIPIDOMICS RESEARCH FACILITY (ALRF)

RESOURCE MOBILZATION
New Funded Projects based on ALRF/FIST Facility

Title of the Project	Principal Investigator	Sponsoring Agency	Sanction No.	Sanctioned Amount (Rs.)
Evaluation of antimycobacterial potential of Unani Drugs Qurs-e-SartanKafoori and Sharbat-e-Ejaz-A Mechanistic Approach	Dr Zeeshan Fatima	GOVERMENT OF INDIA MINISTRY OF AYUSH	Z.28015/227/2015-HPC(EMR)-AYUSH-C	4789970.00
Unraveling the Role of mTORC2 in Regulation of Sphingolipid Biosynthesis in Breast Cancer	Dr Ujjaini Dasgupta	DBT	BT/PR19634/BIC/101/488/2016	5453463.00
Mechanism, evolution, and pharmacology of multidrug resistance in the emerging fungal pathogen Candida auris among Indian cohort of patients	Dr Rajendra Prasad	ICMR	AMR/149/2018-ECD-II	9462600.00
Comprehensive Omics studies to understand the biology of drug resistance Mycobacterium tuberculosis clinical isolates from Arunachal Pradesh	Dr Zeeshan Fatima	DBT	BT/PR23016/NER/95/581/2017	1190000.00
A mass spectrometric approach to unravel the landscape of sphingolipids as major signaling determinants of drug resistance and virulence in emerging human fungal pathogen Candida auris	Dr Rajendra Prasad	DBT	BT/PR38505/MED/29/1513/2020	8425600.00
Insights into the efflux pump arsenal of the emerging pathogen C. auris and its implication in high order of antifungal resistance and virulence	Dr Atanu Banerjee	DBT	BT/PR32349/MED/29/1456/2019	5489236.00
Elucidation of the Role of UDP-Glucose Ceramide Glucosyltransferase (UGCG) in mTORC2-Mediated Regulation of Sphingolipid Biosynthesis	Dr Ujjaini Dasgupta	DBT	BT/PR40413/BRB/10/1922/2020	9810000.00
Comparative Sphingolipid Profiling of Breast Cancer Cell and (TNBC) and Luminal A patients and their Clinicopathological Correlation	Dr Ujjaini Dasgupta	ICMR	5/13/81/2020 NCD-III	4000000.00
Comparative Sphingolipid Profiling of Breast Cancer Cell and Tissue Types	Dr Ujjaini Dasgupta	SERB	ECR/2016/001603	3225000.00

NEW ASSETS CREATED
Equipment purchased from ongoing FIST facility dependent projects:
NEW ASSETS CREATED: Rs.70,46,194.00












- Homogenizer with motor, digital speed indicator, transformer speed regulator
- CO2 Incubator
- 4 degree fridge
- -20 degree freezer
- HPTLC
- Trans blot turbo transfer system
- Real Time PCR (2)
- Microscope (Nikon TCS2)
- Probe sonicator (Omni bead ruptor)
- Shaking water bath
- Cell culture hood (Atlantis V42)
- Refrigerated centrifuge (Eppendorf)
- -80 degree freezer

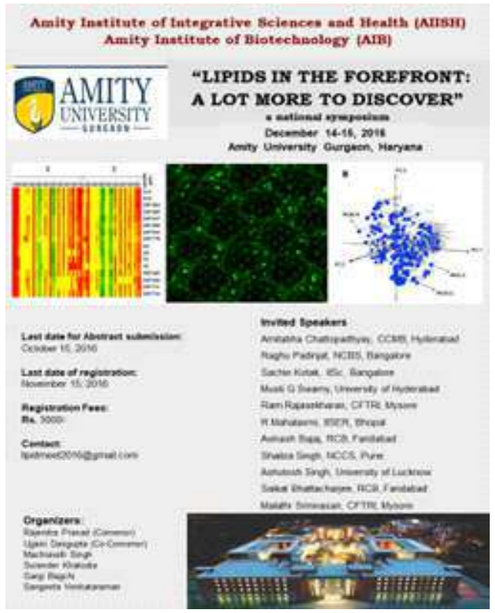
Holding of Regular Workshop/ Symposium/ Webinar:

The following workshops were conducted at ALRF:

As a long-term goal, AUH began talent mapping of National researchers working in lipidomics. In 2016, first lipidomics conference was held in AUH.

1) Lipid Meet 2016:

“Lipids in the forefront:A lot more to discover” was conducted from 14th to 15th December 2016.



FLYER

Group Photograph of Participants.

2) Lipid Meet 2019:

2nd International Symposium "Lipids in the forefront: a lot more to discover”, was conducted on 12-13th December 2019.

FLYER

Group Photograph of Participants.

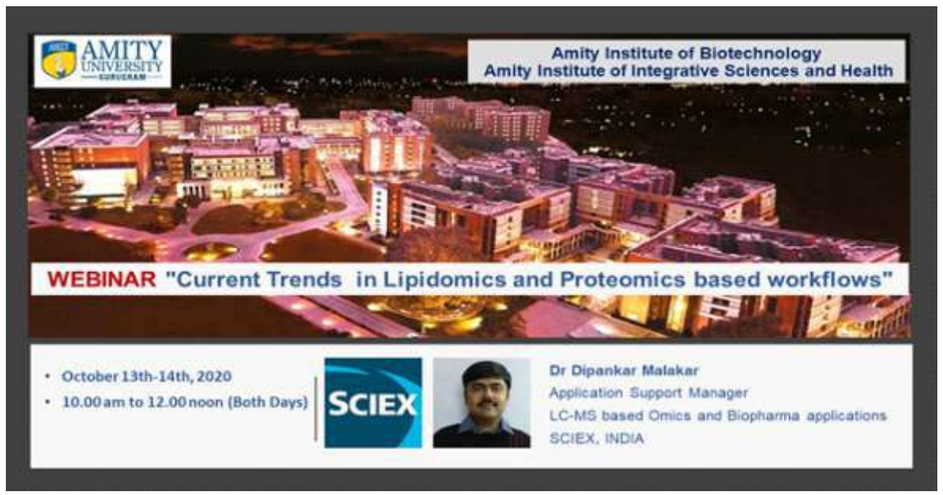
3) Short-term Course on "General Principles of Lipidomics and Proteomics" at Amity Lipidomics Research Facility (ALRF), Amity University Haryana and SCIEX, Gurgaon, Haryana was conducted from 25th to 29th March 2019.

FLYER

The speakers for this workshop include Faraz Rashid, Sciex, Gurgaon, and Dr Ujjaini Dasgupta AIB/AIISH, AUH. Revenue generated from this was Rs. 85902.40

Group Photograph of Participants.

4) "Webinar on Current trends in Lipidomics and Proteomics based workflow"conducted from 13th to 14th October 2020. The speaker for this webinar was Dr Dipankar Malakar, Sciex, India.



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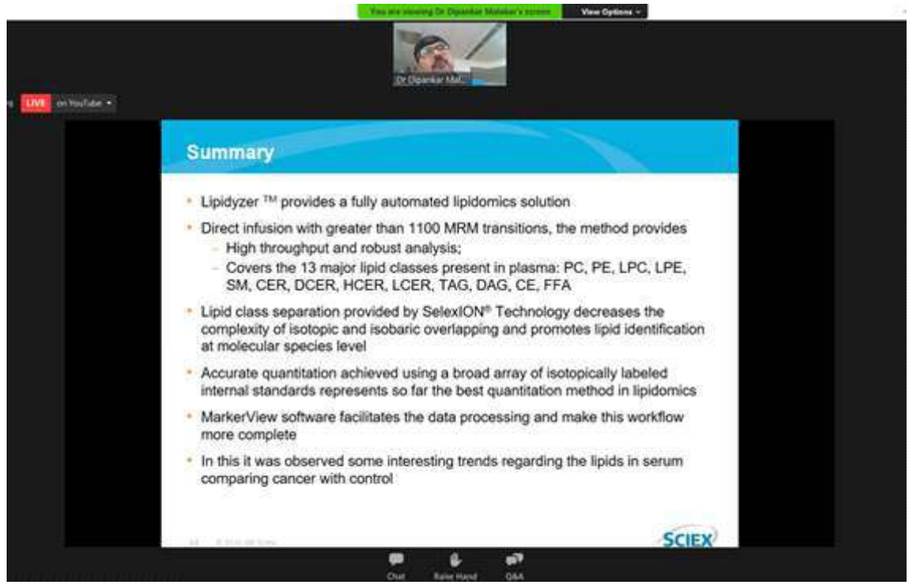

Screenshot of webinar

AMITY LIPIDOMICS RESEARCH FACILITY (ALRF)

ALRF OFFERS THE FOLLOWING SERVICES ON CHARGE BASIS:

Equipment	Analysis	Amity University	Academic& Research Institutions	Private Industries/ other Laboratories
UHPLC Mass spectro-metry	1. ESI-MS based analysis Nominal mass/MW. Determination*	Rs.400 per sample	Rs.800 per sample	Rs.1500 per sample
	2. MS/MS analysis*	Rs. 300 (Per Precursor ion)	Rs.600 (Per Precursor ion)	Rs.1500 (Per Precursor ion)
	3. UPLC-MS analysis (Qualitative)	Rs. 1000 (Per Precursor ion)	Rs.2000 (Per Precursor ion)	Rs.4000 (Per Precursor ion)
	4. UPLC-MS/MS Analysis (Qualitative)	Rs.2500/sample (5 peaks, Rs 200 per additional peak)	Rs.5000/ sample (5 peaks, Rs 300 per additional peak)	Rs.10000/ sample (5 peaks, Rs 400 per additional peak)
	5. UPLC-MS/MS for Targeted analysis of Lipids/Small molecules	Rs 1000/ MRM/analytical lipid unit	Rs 2500/ MRM/analytical lipid unit	Rs 4000/ MRM/analytical lipid unit
	6. UPLC-MS/MS for Targeted analysis of Lipids/Small molecules including data analysis by MultiQuant Software	Rs 1500/ MRM/analytical lipid unit	Rs 4000/ MRM/analytical lipid unit	Rs 5000/ MRM/analytical lipid unit
	7. UPLC-MS/MS for Untargeted analysis of Lipids/Small molecules	Rs.1500/sample	Rs.2500/ sample	Rs.5000/ sample
	8. UPLC-MS/MS for untargeted analysis of Lipids/Small molecules including data analysis by Lipid view Software	Rs.2500/ sample	Rs. 5,000/ sample	Rs.10,000/ sample
For Internal users	Rs 500 per day for internal users provided they bring their own reagents, HPLC column, guard column, solvents, standards, and other consumables. Website: *https://www.amity.edu/gurugram/lipidomics-research-facility.aspx*			

SELECT USERS

The facility is being utilized by various internal and external users; some of them are enlisted below:

- **Rajendra Prasad, Amity University Haryana**
- **Ujjaini Dasgupta, Amity University Haryana**
- **Zeeshan Fatima, Amity University Haryana**
- **Sumukha Hegde, KMC MAHE, Manipal**
- **Divya Yadav, KMC MAHE, Manipal**
- **Deepu Vijayan, Botanical Survey of India**
- **Mohit Kumar, Amity University Haryana**
- **Praveen Kumar, Amity University Haryana**
- **Anshu, Amity University Haryana**
- **Rajlaxmi Yadav, Amity University Haryana**
- **Basharat Ali, Jawaharlal Nehru University**
- **Mohd. Wasi, Jawaharlal Nehru University**
- **Pankaj Sharma, Amity University Haryana**
- **Trishna Pani, Amity University Haryana**
- **Devashish Mehta, Amity University Haryana**
- **Nihal Medatwal, Manipal University**
- **Kajal Rajput, Amity University Haryana**
- **Himanshu Sharma, Amity University Haryana**
- **Dyuti Purkait, Amity University Haryana**
- **Ashutosh Singh, Lucknow University**
- **Saif Hameed, Amity University Haryana**
- **Sudip Majumdar, Amity University Haryana**
- **Nutan Kaushik. Amity University Uttar Pradesh**
- **Sandeep Hans, Amity University Haryana**
- **Judith Berman, Tel Aviv University, Israel**
- **Garima Shahi, Amity University Haryana**
- **Amandeep Saini, Amity University Haryana**
- **Suman Sharma, Amity University Haryana**
- **Sonam Kumari, ICGEB**
- **Syed Bilal Jilani, Amity University Haryana**

PHOTO GALLERY

ALRF/FIST TEAM:

Dr Rajendra Prasad
Director

Dr Ujjaini Dasgupta
Coordinator

Kanchan Pandey
Manager

Kaushavi Cholke
Technical Officer

Amandeep Kaur
Project Manager

Rahul
Laboratory Assistant

AMITY LIPIDOMICS RESEARCH FACILITY (ALRF)

PUBLICATIONS

Chapter 20
Insights into *Candida* Lipids

Rajendra Prasad, Sudhanshu Shukla and Ashutosh Singh

Abstract Although lipid metabolic pathways are fairly well established in yeast, our knowledge of lipid compositional profile, particularly in pathogenic species, is rather limited. Fungal lipids are important on two accounts; first, they possess lipids, particularly sphingolipids, which are unique to *Candida* species and are absent in mammalian host hence are novel drug targets. Second, the functionality of some of the multidrug resistance (MDR) export proteins is dependent upon optimal lipid environment implying their role in clinical drug resistance. The comprehensive high-throughput lipidomics combined with genetic approaches applied to human pathogenic diploid *C. albicans* has started providing insight into mysteries surrounded around this important class of biomolecules. Recent studies already revealed functional interactions between lipids, virulence, and MDR determinants in *Candida*. This chapter reviews some of the recent advances in the field and highlights the role of lipids involved in cross talks between different cellular circuits that impact the acquisition of MDR in *Candida*.

20.1 Introduction

The complexity and dynamic nature of the "lipidome" of eukaryotic cells are increasingly apparent. Taking into account the diversity within many lipid classes created by variability in such factors as acyl chain substitutions, degree of desaturation, hydroxylation, and phosphorylation, the number of molecular species in

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R. Prasad (ed.), *Candida albicans: Cellular and Molecular Biology*,
DOI 10.1007/978-3-319-50409-4_20

Biometals (2019) 32:49–63
https://doi.org/10.1007/s10534-018-0157-8

Altered drug efflux under iron deprivation unveils abrogated MmpL3 driven mycolic acid transport and fluidity in mycobacteria

Rahul Pal · Saif Hameed · Zeeshan Fatima

Received: 15 September 2018 / Accepted: 12 November 2018 / Published online: 15 November 2018

Abstract Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (MTB) is a global threat to human health hence better understanding of the MTB pathogenesis for improved therapeutics requires immediate attention. Emergence of drug-resistant strains has stimulated an urgent need for adopting new strategies that could be implemented to control TB. One of the contributing mechanisms by which MTB evades drug doses is overexpression of drug efflux pumps. Thus blocking or modulating the functionality of efflux pumps represents an attractive approach to combat drug resistance. Iron is a critical micronutrient required for MTB survival and not freely available inside the host. In this study, we demonstrated that iron deprivation impairs drug efflux pump activity and confers synergism for anti-TB drugs in presence of efflux pump inhibitors against MTB. Mechanistic insights revealed that iron deprivation inhibit resistance nodulation division superfamily transporter activity. This was evident from enhanced Nile red accumulation and reduced expression of MmpL3, a transmembrane promising target involved in mycolic acid transport across membrane. Furthermore, iron deprivation led to abrogated MA transport particularly of class methoxy-MA which was confirmed by TLC and mass spectrometry based lipidome analysis. Additionally, iron deprivation leads to enhanced membrane fluidity in MTB. Together, MmpL3 being a promiscuous anti-TB target, metal chelation strategy could be adopted to boost the effectiveness of current anti-TB drug regimes to combat drug resistance TB.

Keywords *Mycobacterium* · Iron · Efflux pump · MmpL3 · Mycolic acid · Membrane fluidity

Introduction

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (MTB) remains one of the important underlying causes of mortality worldwide. Widespread and prolonged deployment of anti-TB agents has led to emergence of drug resistance in MTB, which poses a serious threat to available therapy. Multidrug resistant tuberculosis (MDR-TB) results due to simultaneous resistance towards two frontline anti-TB drugs, isoniazid and rifampicin (Mezwa et al. 2018). Similarly, extensively drug resistant TB (XDR-TB) is caused by MTB strains which are resistant to isoniazid, rifampicin, a fluoroquinolone and one of the three second line

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ORIGINAL ARTICLE

Understanding lipidomic basis of iron limitation induced chemosensitization of drug-resistant *Mycobacterium tuberculosis*

Rahul Pal[1] · Saif Hameed[1] · Parveen Kumar[2] · Sarman Singh[2] · Zeeshan Fatima[1]

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Abstract

Under limited micronutrients condition, *Mycobacterium tuberculosis* (MTB) has to struggle for acquisition of the limited micronutrients available in the host. One such crucial micronutrient that MTB requires for the growth and sustenance is iron. The present study aimed to sequester the iron supply of MTB to control drug resistance in MTB. We found that iron restriction renders hypersensitivity to multidrug-resistant MTB strains against first-line anti-TB drugs. To decipher the effect of iron restriction on possible mechanisms of chemosensitization and altered cellular circuitry governing drug resistance and virulence of MTB, we explored MTB cellular architecture. We could identify non-intact cell envelope, tampered MTB morphology and diminished mycolic acid under iron restricted MDR-MTB cells. Deeper exploration unraveled altered lipidome profile observed through conventional TLC and advanced mass spectrometry-based LC–ESI–MS techniques. Lipidome analysis not only depicted profound alterations of various lipid classes which are crucial for pathogenecity but also exposed leads such as indispensability of iron to sustain metabolic, genotoxic and oxidative stresses. Furthermore, iron deprivation led to inhibited biofilm formation and capacity of MTB to adhere buccal epithelial cells. Lastly, we demonstrated enhanced survival of *Mycobacterium*-infected *Caenorhabditis elegans* model under iron limitation. The present study offers evidence and proposes alteration of lipidome profile and affected virulence traits upon iron chelation. Taken together, iron deprivation could be a potential strategy to rescue MDR and enhance the effectiveness of existing anti-TB drugs.

Keywords *Myocbacterium* · Iron · Lipids · Membrane · Lipidomics · Glyoxylate cycle · Biofilm

Abbreviations

MTB	*Mycobacterium tuberculosis*
MDR	Multidrug resistance
ADC	Albumin dextrose catalase
OADC	Oleic albumin dextrose catalase
2,4 DNP	2,4 dinitrophenol
CFW	Calcoflour white
CV	Crystal violet
INT	Iodonitrotetrazolium chloride
SEM	Scanning electron microscopy
PI	Propidium iodide
DCFDA	2′,7′-dichlorofluorescin diacetate
DAPI	4′,6-diamidino-2-phenylindole
MS	Malate synthase
ICLI	Isocitrate lyase
ROS	Reactive oxygen species
EMB	Ethambutol
RIF	Rifampicin
INH	Isoniazid
STP	Streptomycin
2,2,-BP	2,2, Bipyridyl
FA	Fatty acid
GL	Glycerolipid
GPL	Glycerophospholipid
PK	Polykedide
PR	Prenol
SCL	Saccharolipide

Rahul Pal and Saif Hameed contributed equally.

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Chapter 8
Lipidomics Approaches: Applied to the Study of Pathogenesis in *Candida* Species

Ashutosh Singh, Nitesh Kumar Khandelwal and Rajendra Prasad

Abstract High rate of reported cases of infections in humans caused by fungal pathogens pose serious concern. Potentially these commensal fungi remain harmless to the healthy individuals but can cause severe systemic infection in patients with compromised immune system. Effective drug remedies against these infections are rather limited. Moreover, frequently encountered multidrug resistance poses an additional challenge to search for alternate and novel targets. Notably, imbalances in lipid homeostasis which impact drug susceptibility of *Candida albicans* cells do provide clues of novel therapeutic strategies. Sphingolipids (SPHs) are unique components of *Candida* cells, hence are actively exploited as potential drug targets. In addition, recent research has uncovered that several SPH intermediates and of other lipids as well, govern cell signaling and virulence of *C. albicans*. In this chapter, we highlight the role of lipids in the physiology of *Candida*, particularly focusing on their roles in the development of drug resistance. Considering the importance of lipids, the article also highlights recent high-throughput analytical tools and methodologies, which are being employed in our understanding of structures, biosynthesis, and roles of lipids in fungal pathogens.

Keywords Lipids · Pathogenic fungi · Functions · Mass spectrometry

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RESEARCH ARTICLE

Monoterpenoid Geraniol Improves Anti-mycobacterial Drug Efficiency by Interfering with Lipidome and Virulence of Mycobacteria

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Abstract: ***Background***: Tuberculosis (TB) remains a global infectious disorder for which efficient therapeutics are elusive. Nature is a source of novel pharmacologically active compounds with many potential drugs being derived directly or indirectly from plants, microorganisms and marine organisms.

***Objective*:** The present study aimed to elucidate the antimycobacterial potential of Geraniol (Ger), monoterpene alcohol, against *Mycobacterium smegmatis.*

***Methods*:** Disrupted membrane integrity was studied by membrane permeability assay and PI uptake. Cell surface phenotypes were studied by colony morphology, sliding motility and cell sedimentation rate. Lipidome profile was demonstrated by thin-layer chromatography and liquid chromatography-electrospray ionization mass spectrometry. Amendment in iron homeostasis was assessed by using iron chelator ferrozine and ferroxidase assay while genotoxicity was estimated with EtBr and DAPI staining. Biofilm formation was measured by staining, dry mass and metabolic activity using crystal violet. Cell adherence was examined microscopically and spectrophotometrically.

***Results*:** We found the antimycobacterial activity of Ger to be 500 µg/ml against *M. smegmatis.* Underlying mechanisms revealed impaired cell surface phenotypes. Lipidomics analysis exposed profound decrement of mycolic acids, phosphatidylinositol mannosides and triacylglycerides which are crucial for MTB pathogenicity. We further explored that Ger impairs iron homeostasis and leads to genotoxic stress. Moreover, Ger inhibited the potential virulence attributes such as biofilm formation and cell adherence to both polystyrene surface and epithelial cells. Finally, we have validated all the disrupted phenotypes by RT-PCR which showed good correlation with the biochemical assays.

***Conclusion*:** Taken together, the current study demonstrates the antimycobacterial mechanisms of Ger, which may be exploited as an effective candidate of pharmacological interest.

Keywords: *Mycobacterium*, Anti-TB drugs, Geraniol, Membrane, Lipidome, Biofilm.

1. INTRODUCTION

Mycobacterium tuberculosis (MTB) is a life-threatening human pathogen that causes Tuberculosis (TB). It is estimated that about 10 million populations (5.8 million men, 3.2 million women and 1 million children) suffered from TB in 2017 [1]. The recommended standard anti-TB drugs are - isoniazid (INH), rifampicin (RIF), ethambutol (EMB) and pyrazinamide (PZA) which have potential to sterilize both semi-dormant and actively multiplying mycobacterial bacilli. However, the current anti- TB drugs are becoming less effective due to the emergence of resistant strains of MTB resulting in patients remaining infectious for a longer period and requiring a prolonged course of treatment which has many side effects on human health [2]. In order to impede the problem of multidrug resistance (MDR), the pace of drug discovery needs to be enhanced. Nature represents a rich source of a still undiscovered plethora of compounds that are underutilized and could be better exploited [3]. Geraniol (Ger) is acyclic monoterpene alcohol which occurs in geranium, lemon and many other essential oils such as rose oil, palmarosa oil, citronella oil exhibiting various properties such as anticancer activity [4], anti-inflammatory activity [5] and antimicrobial activity [6]. Previous studies also suggested that Ger demonstrated antimicrobial activity against *Candida albicans*, *Saccharomyces cerevisiae*, *Enterobacter aerogenes, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus agalactiae* and *Bacillus cereus.* In a preliminary study, Rajab *et al.* reported antimycobacterial activity of Ger however, its mechanism of

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Cholic Acid-Derived Amphiphile which Combats Gram-Positive Bacteria-Mediated Infections via Disintegration of Lipid Clusters

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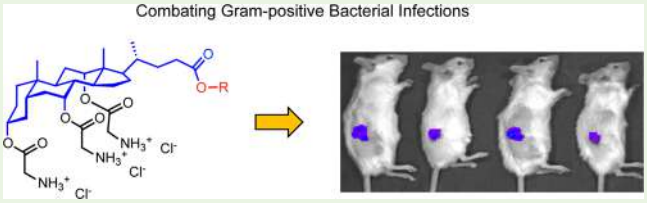
ABSTRACT: Inappropriate and uncontrolled use of antibiotics results in the emergence of antibiotic resistance, thereby threatening the present clinical regimens to treat infectious diseases. Therefore, new antimicrobial agents that can prevent bacteria from developing drug resistance are urgently needed. Selective disruption of bacterial membranes is the most effective strategy for combating microbial infections as accumulation of genetic mutations will not allow for the emergence of drug resistance against these antimicrobials. In this work, we tested cholic acid (CA) derived amphiphiles tethered with different alkyl chains for their ability to combat Gram-positive bacterial infections. In-depth biophysical and biomolecular simulation studies suggested that the amphiphile with a hexyl chain (**6**) executes more effective interactions with Gram-positive bacterial membranes as compared to other hydrophobic counterparts. Amphiphile **6** is effective against multidrug resistant Gram-positive bacterial strains as well and does not allow the adherence of *S. aureus* on amphiphile **6** coated catheters implanted in mice. Further, treatment of wound infections with amphiphile **6** clears the bacterial infections. Therefore, the current study presents strategic guidelines in design and development of CA-derived membrane-targeting antimicrobials for Gram-positive bacterial infections.



KEYWORDS: *antibacterial, bile acids, membrane interactions, MD simulations, antimicrobials*

INTRODUCTION

Infections caused by Gram-positive bacteria cause critical medical conditions such as abscesses, endocarditis, osteomyelitis, and pneumonia.[1] Treatment of these ailments faces extreme challenges due to the emergence of bacterial resistance toward existing antibiotic regimens.[2] Most of the commonly used antibiotics target the essential components of bacterial cellular machinery and become ineffective due to different genetic mutations.[3] Bacterial membranes are considered as the effective target for antimicrobial agents as physical damage of the membranes can directly kill the bacteria and disrupt other cellular functions of bacteria.[4,5] Therefore, the membrane-targeting bactericidal effect of antimicrobials mediated by electrostatic and hydrophobic interactions will not allow the bacteria to develop drug resistance as well.

Antimicrobial peptides (AMPs) are present in all organisms and act against different microbial infections by performing selective interactions with the microbial membranes.[6,7] The

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RESEARCH ARTICLE

Lipidomic Insight of Anticandidal Perillyl Alcohol and Sesamol Induced *Candida* Membrane Disruption: Implications of Lipid Alteration, Impaired Fluidity and Flippase Activity

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Abstract: ***Background*:** Considering the emergence of multidrug resistance (MDR) in prevalent human fungal pathogen, *Candida albicans*, there is parallel spurt in the development of novel strategies aimed to disrupt MDR. The cell envelope of *C. albicans* comprises a wealth of lipid moieties contributing towards long-term survival of pathogen that could be exploited as efficient antifungal target owing to the advancements made in mass spectrometry based lipidomics technology.

***Objective*:** This study aimed to utilize the lipidomics approach to unveil several lipid-associated changes in response to two natural anticandidal compounds perillyl alcohol (PA) and sesamol (Ses).

***Method*:** Lipidomics is performed through ESI-MS, flippase activity by FACS, fluorescence spectrometric analysis is used to assess membrane fluidity.

***Results*:** Lipidomic analyses revealed that phosphatidylcholine (PtdCho) were decreased in the presence of Ses with considerable differences at specie level. Concurrently, we explored increased inward translocation (flip) of fluorophore labelled PtdCho across the plasma membrane attributed to enhanced PtdCho specific flippase activity. A considerable decrement in phosphatidylethanolamine (PtdEtn) leading to altered membrane fluidity was observed in response to PA and Ses. Additionally, we could detect alteration in the levels of phohatidylserine (PtdSer) and phosphatidylglycerol (PtdGro) along with decreased triacylglycerides (TAG). The differential expressions of various lipid biosynthetic pathway genes by RT-PCR corroborated with the lipidomics data. Furthermore, PA and Ses leads to potentiation of membrane targeting drugs (azole and polyene) and displayed additive effect.

***Conclusion*:** Our work offers the basis of further understanding the regulation of lipid homeostasis in *C. abicans* so that better therapeutic targets could be identified to combat MDR.

Keywords: *Candida*, perillyl alcohol, sesamol; lipidomics, cell membrane; phospholipids.

1. INTRODUCTION

The major impediment for efficient therapeutics against the most prevalent human fungal pathogen, *Candida albicans*, is the acquisition of multidrug resistance (MDR) by various mechanisms. Thus, there is an immediate need to look for novel drug targets that could be exploited for effective antifungal therapy. The cell membrane profile and their global or local amendments play an essential role in the typical functioning of the cell. Such processes could be related with the modification of lipid moieties. Although lipid metabolic pathways are fairly well established in yeast but still our knowledge of lipid compositional profile, particularly in pathogenic species, is rather limited considering the fact that among the several causal factors, lipids by far have emerged as one of the critical contributors in the MDR acquisition [1]. Several antifungal drugs target enzymes that are involved in lipid biosynthesis in *Candida*. Membrane lipid metabolism and physical properties appear to be closely linked to MDR in *C. albicans*. For instance, changes in membrane lipid phase and asymmetry affects azole resistance [2]. The drug susceptibility phenotype of *Candida* appears to result from the interplay between drug diffusion and the membrane lipid environment [3, 4]. Lipids are also involved with the MDR mechanisms reflected from many studies where ergosterol plays a major role in the susceptibility of fungal cells to the current therapeutic drugs. In both, the pathogenic fungus *C. albicans* as well as nonpathogenic strain such as *Saccharomyces cerevisiae* the knockout mutants of ergosterol and

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ORIGINAL ARTICLE

Lipidomic insights to understand membrane dynamics in response to vanillin in *Mycobacterium smegmatis*

Sharda Sharma[1] · Saif Hameed[1] · Zeeshan Fatima[1]

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Abstract

Considering the emergence of multidrug resistance (MDR) in prevalent human pathogen, *Mycobacterium tuberculosis* (MTB), there is parallel spurt in development of novel strategies aimed to disrupt MDR. The cell envelope of MTB comprises a wealth of lipid moieties contributing towards long-term survival of pathogen that could be exploited as efficient antitubercular target owing to advancements made in mass spectrometry–based lipidomics technology. This study aimed to utilize the lipidomics approach to unveil several lipid associated changes in response to natural antimycobacterial compound vanillin (Van) in *Mycobacterium smegmatis*, a surrogate for MTB. Lipidomic analyses revealed that that Van alters the composition of fatty acid (FA), glycerolipid (GL), glycerophospholipid (GP), and saccharolipids (SL). Furthermore, Van leads to potentiation of ampicillin and displayed additive effect. The differential expressions of various lipid biosynthetic pathway genes by RT-PCR corroborated with the lipidomics data. Lastly, we demonstrated enhanced survival of *Mycobacterium*-infected *Caenorhabditis elegans* model in presence of Van. Thus, lipidomics approach provided detailed insight into mechanisms of membrane disruption by Van in *Mycobacterium smegmatis*. Our work offers the basis of further understanding the regulation of lipid homeostasis in MTB so that better therapeutic targets could be identified to combat MDR.

Keywords *Mycobacterium* · Vanillin · Lipids · Cell wall · Fatty acid · Glycerolipids · Glycerophospholipids

Introduction

The evolution of drug-resistant *Mycobacterium tuberculosis* (MTB) has established severe complications that are difficult to treat and generated considerable concern for developing effective strategies for the control of tuberculosis (TB). Although the current drug susceptibility testing is quite accurate and efficient, it is time-consuming. Identification of diagnostic biomarkers is, therefore, necessary to discriminate between infection from drug-resistant and drug-susceptible strains. One strategy that helps to effectively control TB is to understand the function of lipids that mycobacteria use to manipulate host cellular defenses. MTB has unique cell envelope architecture comprising several lipids between the outer and inner membrane which account for much of its impermeability to anti-TB drugs and confer unique staining properties to MTB (Jackson 2014).

The recent introduction of high-throughput analyses of lipids is accelerating our ability to analyze MTB lipid metabolism and signaling and the factors that regulate those pathways (Pal et al. 2017; Sharma et al. 2018). Several categories of lipid are present in MTB, e.g., fatty acids (FA), glycerolipids (GL), glycerophospholipids (GP), prenol (PR), polyketides (PK), and saccharolipids (SL). The outer membrane and capsular lipids of MTB play important roles in host–pathogen interactions. The innermost layer is the plasma membrane that seems typical of bacterial membrane while outside the plasma membrane is a massive cell wall core comprised of peptidoglycan (PG), in covalent attachment via phosphoryl-*N*-acetylglucosaminosyl-rhamnosyl linkage units with the heteropolysaccharide arabinogalactan (AG), which in turn is esterified at its non-reducing ends to α-alkyl, β-hydroxy long-chain (C_{60}-C_{90}) mycolic acids. The cell wall core, also referred to as the mycolyl arabinogalactan-peptidoglycan (mAGP) complex is required for

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A Localized Chimeric Hydrogel Therapy Combats Tumor Progression through Alteration of Sphingolipid Metabolism

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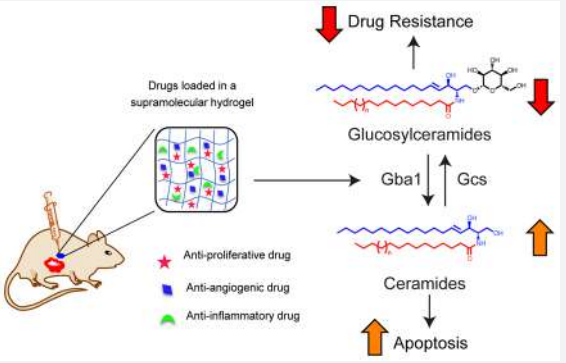
Supporting Information

ABSTRACT: Rapid proliferation of cancer cells assisted by endothelial cell-mediated angiogenesis and acquired inflammation at the tumor microenvironment (TME) lowers the success rate of chemotherapeutic regimens. Therefore, targeting these processes using localized delivery of a minimally toxic drug combination may be a promising strategy. Here, we present engineering of a biocompatible self-assembled lithocholic acid-dipeptide derived hydrogel (TRI-Gel) that can maintain sustained delivery of antiproliferating doxorubicin, antiangiogenic combretastatin-A4 and anti-inflammatory dexamethasone. Application of TRI-Gel therapy to a murine tumor model promotes enhanced apoptosis with a concurrent reduction in angiogenesis and inflammation, leading to effective abrogation of tumor proliferation and increased median survival with reduced drug resistance. In-depth RNA-sequencing analysis showed that TRI-Gel therapy induced transcriptome-wide alternative splicing of many genes responsible for oncogenic transformation including sphingolipid genes. We demonstrate that TRI-Gel therapy targets the reversal of a unique intron retention event in *β*-glucocerebrosidase 1 (*Gba1*), thereby increasing the availability of functional Gba1 protein. An enhanced Gba1 activity elevates ceramide levels responsible for apoptosis and decreases glucosylceramides to overcome drug resistance. Therefore, TRI-Gel therapy provides a unique system that affects the TME via post-transcriptional modulations of sphingolipid metabolic genes, thereby opening a new and rational approach to cancer therapy.



INTRODUCTION

Tumor microenvironment (TME) consists of rapidly proliferating cancer cells infiltrated by different host cell types like vascular endothelial cells, macrophages, tumor-associated fibroblasts, and other immune cells.[1] Intercellular communications occurring through a network of cytokines, chemokines, growth factors, and matrix remodeling enzymes generate a conducive environment for cancer cells to proliferate, invade, metastasize, and develop drug resistance.[2] Angiogenesis and inflammation induced by directed migration of endothelial cells and immune cells trigger an immunosuppressive and pro-proliferative niche at the tumor site.[2] Such a programmed neoplastic transformation reduces the efficacy of chemotherapeutic drugs and

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Nanomaterial-Assisted Mass Spectrometry: An Evolving Cutting-Edge Technique

19

Ashutosh Singh, Nitin Bhardwaj, and Rajendra Prasad

Abstract

In the last decade, the area of "omics research" has received tremendous recognition and found significances in the field of biomedicine. And so did develop the technologies for analyzing various kinds of biomolecules. The advances made in omics tools are quite diverse and advanced. In this chapter, we give an overview of two most common approaches used for the analysis of biomolecules, namely, electrospray ionization and matrix-assisted laser desorption/ionization (MALDI) mass spectrometry. The conventional MALDI approach for biomolecular analysis relies on organic matrices for ionization of analytes, which have several disadvantages in analysis of small molecules. Here we discuss the types and application of nanomaterials in laser desorption/ionization mass spectrometry in the analysis of biomolecules. Additionally, examples of nanomaterial-assisted mass spectrometry imaging are discussed. Together this chapter provides insights into mass spectrometry and significance of nanomaterials in analysis of biomolecules, which have large-scale implications in the field of biomedicine.

Keywords

Electrospray ionization · Matrix-assisted laser desorption/ionization · Biomolecules · Mass spectrometry · Nanoparticles

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Chapter 1

Background of Membrane Lipids

Ashok Kumar, Atanu Banerjee, Ashutosh Singh, and Rajendra Prasad

Abstract

Lipids are a unique group of molecules that universally exist in both prokaryotes and eukaryotes; however, they were least investigated biomolecules owing to their water-insoluble nature. However, this scenario has been changing in the last few decades of intensive research which unraveled diverse roles played by them in a wide variety of biological processes in all spectrum of life. Notwithstanding a common footprint of lipids that exists in most organisms, there are specific lipid molecules, which are characteristic of a system. Coinciding with the development of separation and high-throughput analytical tools, we are able to detect minor lipids which otherwise remained undetected. We now know that each type of phosphoglycerides or sphingolipids is enriched with a host of molecular species imparting additional dynamism to lipid composition. These lipid changes regulate membrane homeostasis, which in turn affects the physiological functions. This chapter provides a background of lipids that are present in biological systems. Since there exists a vast amount of literature on lipid metabolism of various organisms, we will only limit our discussion to yeast systems.

Keywords Lipids, Membrane, Functions

1 Introduction

Lipids are well known as a source of biofuel and as membrane structural components; however, these sparingly water-soluble molecules have recently come to prominence due to the discovery of their multidimensional roles extending from human diseases and infections caused by microbial and fungal organisms [1]. This has fueled greater momentum in lipid research, well supported by the advancement of analytical tools including high-throughput mass spectrometry-based advanced techniques presenting high resolution and detection of lipids which otherwise remained undetected due to their lower content [2]. Among eukaryotic models for unveiling multiple roles of lipids, budding yeast *Saccharomyces cereviseae* has been a preferred model. The genetic flexibility provided by this haploid yeast has enabled us to unknot various facets of lipids. As a result, a fairly good knowledge concerning the spectrum of lipid composition, and regulatory circuitry governing

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Sphingolipidomics of drug resistant *Candida auris* clinical isolates reveal distinct sphingolipid species signatures

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ABSTRACT

Independent studies from our group and others have provided evidence that sphingolipids (SLs) influence the antimycotic susceptibility of *Candida* species. We analyzed the molecular SL signatures of drug-resistant clinical isolates of *Candida auris*, which have emerged as a global threat over the last decade. This included Indian hospital isolates of *C. auris*, which were either resistant to fluconazole (FLC^R) or amphotericin B (AmB^R) or both drugs. Relative to *Candida glabrata* and *Candida albicans* strains, these *C. auris* isolates were susceptible to SL pathway inhibitors such as myriocin and aureobasidin A, suggesting that SL content may influence azole and AmB susceptibilities. Our analysis of SLs confirmed the presence of 140 SL species within nine major SL classes, namely the sphingoid bases, Cer, αOH-Cer, dhCer, PCer, αOH-PCer, αOH-GlcCer, GlcCer, and IPC. Other than for αOH-GlcCer, most of the SLs were found at higher concentrations in FLC^R isolates as compared to the AmB^R isolates. SLs were at intermediate levels in FLC^R + AmB^R isolates. The observed diversity of molecular species of SL classes based on fatty acyl composition was further reflected in their distinct specific imprint, suggesting their influence in drug resistance. Together, the presented data improves our understanding of the dynamics of SL structures, their synthesis, and link to the drug resistance in *C. auris*.

1. Introduction

Increasing antimicrobial resistance in pathogenic fungi is becoming a global health threat and eroding our ability to control fungal infections with a limited armamentarium of antifungals [1]. Most of the fungal infections associated with significant mortality and antimicrobial resistance are triggered by opportunistic human fungal pathogens [1,2]. The major pathogens, *Candida albicans*, *Aspergillus fumigatus*, and *Cryptococcus neoformans*, may survive in anatomically distinct locations within the host and are capable of fostering deep-seated infections in patients with compromised immune systems [3]. In contrast to the common *C. albicans*, non-albicans *Candida* (NAC) species are evolving as problematic drug resistance pathogens [1]. The recent emergence of multiple drug-resistant *Candida auris* clades within a short

Abbreviations: MDR, multidrug resistance; NAC, non-albicans *Candida*; FLC, fluconazole; AmB, amphotericin B; MS, mass spectrometry; SLs, sphingolipids; Liquid chromatography-tandem mass spectrometry, LC-MS/MS; FLC^R, FLC-resistant; AmB^R, AmB-resistant; FLC^R + AmB^R, both FLC and AmB resistant; MIPC, mannosyl-inositol-phosphoceramides; $M(IP)_2C$, mannosyl-diinositol-phosphoceramides; PDREs, Pdr1/Pdr3 response elements; PCA, principal component analysis; MYR, myriocin; AbA, aureobasidin A; SPT, serine palmitoyl-CoA transferase; IPC, inositolphosphorylceramide; GlcCer, glucosylceramide; DHS, dihydrosphingosine; SPH, sphingosine; S1P, sphingosine-1-phosphate; DHS1P, dihydrosphingosine-1-phosphate; PHS, phytosphingosine; PHS1P, phytosphingosine-1-phosphate; Glucosyl-SPH, glucosyl sphingosine; dhCer, dihydroceramide; Cer, ceramides; αOH-Cer, αhydroxy ceramides; PCer, phytoceramide; αOH-PCer, αhydroxy phytoceramide; αOH-GlcCer, αhydroxy glucosylceramide; IPCs, inositol phosphoryl ceramides

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RESEARCH ARTICLE

A detailed lipidomic study of human pathogenic fungi *Candida auris*

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One sentence summary: The present study determines the lipid composition of *Candida auris* and highlights alterations in lipids that may be correlated to high drug resistance in fungi.

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ABSTRACT

The present study is an attempt to determine the lipid composition of *Candida auris* and to highlight if the changes in lipids can be correlated to high drug resistance encountered in *C. auris*. For this, the comparative lipidomics landscape between drug-susceptible (CBS10913T) and a resistant hospital isolate (NCCPF_470033) of *C. auris* was determined by employing high throughput mass spectrometry. All major groups of phosphoglycerides (PGL), sphingolipids, sterols, diacylglycerols (DAG) and triacylglycerols (TAG), were quantitated along with their molecular lipid species. Our analyses highlighted several key changes where the NCCPF_470033 showed an increase in PGL content, specifically phosphatidylcholine, phosphatidylglycerol, phosphatidylserine, phosphatidylinositol, and phosphatidylethanolamine; odd chain containing lipids and accumulation of 16:1-DAG and 16:0-DAG; depletion of 18:1-TAG and 18:0-TAG. The landscape of molecular species displayed a distinct imprint between isolates. For example, the levels of unsaturated PGLs, contributed by both odd and even-chain fatty acyls were higher in resistant NCCPF_470033 isolate, resulting in a higher unsaturation index. Notwithstanding, several commonalities of lipid compositional changes between resistant *C. auris* and other *Candida* spp., the study could also identify distinguishable changes in specific lipid species in *C. auris*. Together, the data highlights the modulation of membrane lipid homeostasis associated with drug-resistant phenotype of *C. auris*.

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Hydrogel-mediated delivery of celastrol and doxorubicin induces a synergistic effect on tumor regression *via* upregulation of ceramides†

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The release of anticancer drugs in systemic circulation and their associated toxicity are responsible for the poor efficacy of chemotherapy. Therefore, the identification of new chemotherapeutic combinations designed to be released near the tumor site in a sustained manner has the potential to enhance the efficacy and reduce the toxicity associated with chemotherapy. Here, we present the identification of a combination of doxorubicin, a DNA-binding topoisomerase inhibitor, with a naturally occurring triterpenoid, celastrol, that induces a synergistic effect on the apoptosis of colon cancer cells. Hydrogel-mediated sustained release of a combination of doxorubicin and celastrol in a murine tumor model abrogates tumor proliferation, and increases the median survival with enhanced apoptosis and concurrent reduction in proliferation. Sphingolipid profiling (LC-MS/MS) of treated tumors showed that the combination of celastrol and doxorubicin induces global changes in the expression of sphingolipids with an increase in levels of ceramides. We further demonstrate that this dual drug combination induces a significant increase in the expression of ceramide synthase 1, 4, and 6, thereby increasing the level of ceramides that contribute to the synergistic apoptotic effect. Therefore, hydrogel-mediated localized delivery of a combination of celastrol and doxorubicin provides a new therapeutic combination that induces a sphingolipid-mediated synergistic effect against colon cancer.

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Introduction

Cancer chemotherapy is often challenged with low efficacy and high toxicity of anticancer drugs due to their lack of specificity for cancer cells leading to poor patient survival.[1] Therefore, combination therapy is usually preferred in clinical settings as it helps in reducing the toxicity owing to the lower dosage of chemotherapeutics.[2] Combination therapies also provide advantages of high efficacy, enhanced patient survival, and ability to combat drug resistance as a combination of drugs can modulate multiple signalling pathways in cancer cells.[3] However, numerous challenges are associated with the use of combination therapy like poor knowledge of an appropriate combination of drugs and their dosage, bioavailability of drugs in the desired ratio at the tumor site, their non-specific targeting, varying pharmacokinetics among different drugs, and lack of understanding of their mode of action.[4]

Many natural products like taxanes have been clinically approved for cancer treatment in combination with other therapeutic regimens.[5] Celastrol (CEL), a quinine methide triterpenoid extracted from *Tripterygium wilfordii* Hook. f., is a traditional Chinese medicine.[6,7] Recent studies have shown that CEL can enhance leptin sensitivity,[8] and can also activate the heat shock factor 1 (HSF1) that enhances the energy expenditure and mitochondrial functions for the treatment of obesity.[9] CEL is known to inhibit cancer cell proliferation by inducing the expression of pro-apoptotic proteins like Bax and cytochrome c, and by enhancing the Bax/Bcl-2 ratio.[10,11] CEL reduces the colon tumor progression where CEL-mediated activation of LKB1 activates AMPKα and phosphorylates YAP, leading to the degradation of β-catenin.[12] Administration of CEL also inhibits ulcerative colitis-induced colorectal cancer by preventing the upregulation of β-catenin, and downregulating the expression of inflammatory cytokines.[13] Therefore, a

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Rec A disruption unveils cross talk between DNA repair and membrane damage, efflux pump activity, biofilm formation in *Mycobacterium smegmatis*

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ABSTRACT

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (MTB) has emerged in recent decades as one of the leading causes of mortality worldwide. The burden of TB is alarmingly high, with one third affected global population as reported by WHO. Short-course treatment with an antibiotic is a powerful weapon to treat infection of susceptible MTB strain, however; MTB has developed resistance to anti-TB drugs, which is an escalating global health crisis. Thus there is urgent need to identify new drug targets. RecA is a 38 kilodalton protein required for the repair and maintenance of DNA and regulation of the SOS response. The objective of this study is to understand the effect of disruption of RecA gene (deletion mutant ΔdisA from previous study) in a surrogate model for MTB, *Mycobacterium smegmatis*. This study demonstrated that disruption of RecA causes enhanced susceptibility towards rifampicin and generation of ROS leading to lipid peroxidation and impaired membrane homeostasis as depicted by altered cell membrane permeability and efflux pump activity. Mass spectrometry based lipidomic analysis revealed decreased mycolic acid moieties, phosphatidylinositol mannosides (PIM), Phthiocerol dimycocerosate (DIM). Furthermore, biofilm formation was considerably reduced. Additionally, we have validated all the disrupted phenotypes by RT-PCR which showed a good correlation with the biochemical assays. Lastly, RecA mutant displayed reduced infectivity in *Caenorhabditis elegans* illustrating its vulnerability as antimycobacterial target. Together, present study establishes a link between DNA repair, drug efflux and biofilm formation and validates RecA as an effective drug target. Intricate studies are needed to further understand and exploit this therapeutic opportunity.

1. Introduction

Despite the improvement of tuberculosis (TB) regimen, TB remains the second public severe health problem and a leading cause of death due to an infectious disease after HIV infection [51]. The treatment of TB caused by *Mycobacterium tuberculosis* (MTB) requires at least six months duration, but multidrug-resistant MTB entails second-line drugs that are generally toxic for a longer period usage [36]. Currently, the main obstacle in TB research is limited understanding of the mechanisms by which MTB evades both the host immune response and the emergence of multidrug resistance (MDR). Hence we need to identify novel drug targets to be employed in therapeutic strategies.

The RecA proteins are structurally conserved among eubacteria and archaea [37]. However, the functional connection among RecA proteins is poorly implicit. In comparison to the most-studied *Escherichia coli* RecA, which is essential for several processes related to DNA metabolism, MTB RecA displays many unique, distinctive features [11,29]. In vitro studies have revealed that the MTB RecA protein exhibits numerous differences from other bacterial RecA homologs. Apart from *E. coli* RecA (EcRecA), homologous pairing and strand exchange promoted by MTB RecA (MtRecA) is greatly dependent on the pH of the medium [48]. The MtRecA protein also displays a reduced affinity for ATP and reduced efficiency of ATP hydrolysis [19]. First time [10] function of the MTB RecA protein in DNA repair and integration of exogenous nucleic acids was demonstrated by its ability to fully restore the phenotype of *M. smegmatis* RecA mutants. RecA also has a regulatory function in response to DNA damage, due to the ability of nucleoprotein filament created on regions of single-stranded DNA to stimulate the

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Alternative splicing of *ceramide synthase 2* alters levels of specific ceramides and modulates cancer cell proliferation and migration in Luminal B breast cancer subtype

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Abstract

Global dysregulation of RNA splicing and imbalanced sphingolipid metabolism has emerged as promoters of cancer cell transformation. Here, we present specific signature of alternative splicing (AS) events of sphingolipid genes for each breast cancer subtype from the TCGA-BRCA dataset. We show that *ceramide synthase 2* (*CERS2*) undergoes a unique cassette exon event specifically in Luminal B subtype tumors. We validated this exon 8 skipping event in Luminal B cancer cells compared to normal epithelial cells, and in patient-derived tumor tissues compared to matched normal tissues. Differential AS-based survival analysis shows that this AS event of *CERS2* is a poor prognostic factor for Luminal B patients. As Exon 8 corresponds to catalytic Lag1p domain, overexpression of AS transcript of *CERS2* in Luminal B cancer cells leads to a reduction in the level of very-long-chain ceramides compared to overexpression of protein-coding (PC) transcript of *CERS2*. We further demonstrate that this AS event-mediated decrease of very-long-chain ceramides leads to enhanced cancer cell proliferation and migration. Therefore, our results show subtype-specific AS of sphingolipid genes as a regulatory mechanism that deregulates sphingolipids like ceramides in breast tumors, and can be explored further as a suitable therapeutic target.

Introduction

Sphingolipids help in maintaining the structural integrity of cell membranes, and also aid in numerous signaling processes in response to different stimuli[1–3]. Dysregulation of the sphingolipid pathway is one of the important contributing factors for breast cancer pathogenesis as it is implicated in various aspects of cancer initiation, progression, invasion, metastasis, and drug resistance[4,5]. Any alteration in the enzymes regulating the expression of sphingolipids play a vital role in cancer cell survival and apoptosis[6]. Therefore, dynamic metabolic interconversions among sphingolipids, attuning the cellular signaling mechanisms in cancer cells, make them a desirable yet challenging target for cancer therapy[7,8].

Ceramides are key precursors of complex sphingolipids that are synthesized via de novo as well as through salvage pathway. De novo pathway involves conjugation of specific fatty acyl chains to sphinganine using ceramide synthases, whereas salvage pathway involves degradation of sphingomyelins and complex sphingolipids to ceramides[9]. Human breast cancer tissues usually have high expression of ceramide species as compared to normal tissue samples[10]. There is an increased expression of

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A hydrogel-based implantable multidrug antitubercular formulation outperforms oral delivery†

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We present a non-immunogenic, injectable, low molecular weight, amphiphilic hydrogel-based drug delivery system (TB-Gel) that can entrap a cocktail of four front-line antitubercular drugs, isoniazid, rifampicin, pyrazinamide, and ethambutol. We showed that TB-Gel is more effective than oral delivery of the combination of four drugs in reducing the mycobacterial infection in mice. Results show that half the dose of chemotherapeutic drugs is sufficient to achieve a comparable therapeutic effect to that of oral delivery.

Tuberculosis is a highly contagious infection of the lungs caused by *Mycobacterium tuberculosis* (*M. tuberculosis*)[1] and is one of the leading causes of death worldwide.[2] One-third of the global population is infected with *M. tuberculosis*, and ~10 million new tuberculosis cases were reported in 2019 with ~1.4 million deaths.[2] Although there is only a 5% mortality rate among patients under directly observed therapy and active TB control programs, the mortality rate is 2–10-fold higher in poorly executed programs.[2,3] Over time, it has been established that tuberculosis cannot be treated with a single drug as *M. tuberculosis* inevitably develops multiple-drug resistance.[4,5] Therefore, treatment of tuberculosis comprises a combination of four chemotherapeutic drugs, rifampicin (RIF), isoniazid (INH), pyrazinamide (PYZ), and ethambutol (EMB) daily for two months in the initial phase, followed by a continuation phase of RIF and INH for four months.[4,5]

Patients' non-compliance to the treatment is one of the major challenges for low cure rates despite using an effective multiple drug regimen.[6,7] Therefore, there is a need for drug delivery vehicles that can sustain the release of a combination of antitubercular drugs. Low permeability of these drugs across the gastrointestinal barriers and long treatments are also responsible for toxicity associated with these chemotherapeutic regimens like gastrointestinal problems, impaired vision due to EMB, jaundice due to INH, RIF and PYZ, and itching and burning sensations in the limbs due to INH.[8–10] These adverse side effects are responsible for discontinuation of the treatment, leading to the emergence of multiple drug resistance. Therefore, nanoformulations consisting of liposomes, solid lipid nanoparticles, polymeric micelles, silica nanoparticles, and gold nanoparticles have been employed to deliver the antitubercular drugs.[11–20] However, none of the studies until now have reported an implantable delivery system that can maintain sustained release of a combination of these chemotherapeutic drugs.

Hydrogels are the supramolecular assembly of amphiphiles that hold a large amount of water, and supramolecular aggregation in hydrogels is assisted by hydrophobic and electrostatic interactions.[21] Low molecular weight hydrogels (LMWHs) provide numerous advantages over polymeric hydrogels due to their easy synthesis, biodegradable nature, low immunogenicity, and easy modulation of supramolecular interactions through chemical modifications.[22] Recent studies have shown the impact of LMWHs as potential reservoirs for chemotherapeutic drugs as they can maintain slow and sustained release of chemotherapeutic drugs for ailments like inflammatory bowel disease,[23] arthritis,[24] cancer,[25] and allograft transplantation.[26] Therefore, we hypothesized that entrapment of four antitubercular drugs in an injectable, non-immunogenic, and biodegradable hydrogel can allow the direct release of these drugs in systemic circulation avoiding

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Mechanistic insights into the antimycobacterial action of unani formulation, Qurs Sartan Kafoori

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ABSTRACT

Background and aim: Tuberculosis (TBC) is a deadly disease and major health issue in the world. Emergence of drug resistant strains further worsens the efficiency of available anti-TBC drugs. Natural compounds and particularly traditional medicines such as Unani drugs are one of the promising alternatives that have been widely used nowadays. This study aims to evaluate the efficacy of unani drug Qurs-e-Sartan Kafoori (QSK) on *Mycobacterium tuberculosis* (MTB).

Experimental procedures: Drug susceptibilities were estimated by broth microdilution assay. Cell surface integrity was assessed by ZN staining, colony morphology and nitrocefin hydrolysis. Biofilms were visualized by crystal violet staining and measurement of metabolic activity and biomass. Lipidomics analysis was performed using mass spectrometry. Host pathogen interaction studies were accomplished using THP-1 cell lines to estimate cytokines by ELISA kit, apoptosis and ROS by flow cytometry.

Results: QSK enhanced the susceptibilities of isoniazid and rifampicin and impaired membrane homeostasis as depicted by altered cell surface properties and enhanced membrane permeability. In addition, virulence factor, biofilm formation was considerably reduced in presence of QSK. Lipidomic analysis revealed extensive lipid remodeling. Furthermore, we used a THP-1 cell line model, and investigated the immunomodulatory effect by estimating cytokine profile and found change in expressions of TNF-α, IL-6 and IL-10. Additionally, we uncover reduced THP-1 apoptosis and enhanced ROS production in presence of QSK.

Conclusion: Together, this study validates the potential of unani formulation (QSK) with its mechanism of action and attempts to highlight its significance in MDR reversal.

1. Introduction

Infectious diseases are the main cause of human death worldwide and Tuberculosis (TBC) which is an ancient disease has affected mankind for more than 4,000 years.[1] TB remains a leading cause of morbidity and mortality in developing countries. The estimated number of infections by *Mycobacterium tuberculosis* (MTB), causative agent of TBC is one third of the world population with 8–9 million new TBC cases each year and about 1–2 million yearly deaths.[2] According to report of WHO in 2018, the 30 high TBC burden countries accounted for 87% of new TBC cases among which 8 countries contributed for two thirds of the total, with India leading the count. The report also described a total of 1.5 million people who died from TBC in 2018 and 10 million people fell ill with TBC worldwide which included 5.7 million men, 3.2 million women and 1.1 million children (including 251,000 people with HIV).[3] Worldwide, TBC is one of the top 10 causes of death and the leading cause from a single infectious agent (above HIV/AIDS).[4] The situation get worsen with the emergence of multidrug-resistant TBC (MDR-TBC) which is a public health crisis and a health security threat. WHO estimated around 484,000 new cases with resistance to rifampicin, the most effective first-line drug of which 78% had MDR-TBC. The MDR-TBC burden largely falls on 3 countries India, China and the Russian Federation which together accounts

Abbreviations: Qurs sartan kafoori, (QSK); Isoniazid, (INH); Rifampicin, (RIF); Streptomycin, (STP); Ethambutol, (EMB); Reactive oxygen species, (ROS); fatty acid, (FA); glycerolipids, (GL); glycerophospholipids, (GPL).

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