YENEPOYA
(DEEMED TO BE UNIVERSITY)
Recognized under Sec 3(A) of the UGC Act 1956
Accredited by NAAC with 'A' Grade

Details of the Collaborative Activity

2020-21

Name of the Collaborating Institute: Manipal Academy of Higher Education, Manipal, Karnataka, India.

Name of the Collaborating Department: Yenepoya Research Center

Joint Research Projects Granted by ICMR:

1. Understanding the clomiphene citrate-induced changes in secretary epithelial cells of human fallopian tube: 2020-21

   Collaborators: Dr. Keahsava Prasad, YRC and Dr. Guruprasad K, MAHE

Multicentric Study

1. Estimation of sexual dimorphism using adult human mandibles of south Indian origin: a multi centric study: Nov 2020

Collaborators: Dr. Meera Jacob from YMC and Dr. Vrinda Hari Ankolekar from KMC

Joint research publications

- Vashasnatha M, Yashwant S, Patis S, Riaz A et al. Molecular alterations in oral cancer between tobacco chewers and smoker using serum proteomics. *Cancer Biomarkers* 2021; 31: 361-73.
- K Sapna, M Tarique, A Asiamma, TN Ravi Kumar, V Shashidhar, AB Arun, Prasad KS. Early detection of Leptospirosis using Anti-LipL32 carbon nanotube immunofluorescence probe. *Journal of Bioscience and Bioengineering* 2020.130(4),424
- Das R, Ivanisenko VA, Anashkina AA, Upadhyai P. The story of the lost twins: decoding the genetic identities of the Kumhar and Kurcha populations from the Indian subcontinent. *BMC Genetics*. 2020; 21(1):1-1.
- Parvez K, AB Arun, Rekha PD, Almutiri RHS, Zeyadi AZ, Budihal SV. High mutation rate leads to fitness loss for Corona virus quasispecies. *Coronaviruses*. 2021: e310821191321.

ATTESTED

Dr. Gangadhara Somayaji K S
Registrar
Yenepoya (Deemed to be University)
University Road, Deralakatte
Mangalore 575 018, Karnataka.

Collaborative Agreement

This Collaborative Agreement (referred to as "CA" in the future) is entered into on this 10 day of November 2020.

BETWEEN

Dr. T. S. Keshava Prasad, Professor and Deputy Director, Center for Systems Biology and Molecular Medicine (CSBMM), Yenepoya Research Centre, an entity of Yenepoya (Deemed to be University) situated at University Road, Deralakatte Mangalore 575018, India, hereinafter referred to as "First Party"

And

Dr. Guruprasad Kalthur, Professor, Clinical Embryology, Kasturba Medical College, Manipal Academy of Higher Education, Manipal- 576 104, Karnataka, India, hereinafter referred to as "Second Party".

Dr. T. S. Keshava Prasad and Guruprasad Kalthur will hereinafter be referred to collectively as "Participants" or individually as "Participant", as applicable.

1.1 Background. Dr. Guruprasad Kalthur and Dr. Keshava Prasad have been planning to collaborate for the last two years. They have recently obtained an ICMR research grant entitled "Understanding the clomiphene citrate-induced changes in secretory epithelial cells of the human fallopian tube" (ICMR submission id 2020-3410). Therefore, they are writing this collaborative agreement.

1.2 Objectives. This CA reflects the Participants' sincere and genuine intentions to collaborate in this specific project -"Understanding the clomiphene citrate-induced changes in secretory epithelial cells of the human fallopian tube".

The purpose of this CA is to advance the collaborative ideas and objectives of the Participants as they relate to this specific study, and enable each of the Participants to pursue the research activities and tasks set out in Article II of this CA.

ARTICLE II

(Scope of Collaboration)

2.1 **General Scope.** Each Participant will foster a collaborative research relationship with the other Participant.

2.2 **Specific Research Activities.** The Participants intend to collaboratively pursue the following research activities and goals:

2.2.1. Proteomic analysis of rat and human sperms exposed to clomiphene citrate in different conditions to identify altered signaling pathways and downstream signaling molecules

2.2.2 Proteomic analysis of the effect of clomiphene citrate on the function of human fallopian tube secretory epithelial cells

2.2.3 To identify CC-induced variation in secretome profile of human fallopian tube secretory cells and to assess the influence of secretome on the sperm function under vitro conditions

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Dr.Gangadhara Somayaji K.S.
Registrar
Yenepoya(Deemed to be University)
University Road, Deralakatte
Mangalore- 575 018, Karnataka

2.2.4 Train each other's students in proteomics

2.3. Human Samples:

2.3.1. The Second Party will obtain human clinical samples for the study after following appropriate Institutional Ethics Committee (IEC) guidelines and obtaining approval of the IEC.

2.3.2. The First Party will carry out only proteomic analysis of clinical samples provided by the Second Party.

2.3.2. The Second Party will provide the human clinical samples to the First Party to carry out proteomic analysis only after anonymizing the sample source.

2.3.3. The First Party will not recruit any clinical samples to this study.

2.3 Further Agreements. This document shall delineate the Participants rights and obligations, will address, among other things, sources of funding and intellectual property rights, and be signed by both Participants, before commencing any research activity

2.3.1 Each Participant will abide by all regulations, policies and procedures of their Institutions regarding the disclosing and handling of intellectual property, developed technologies, and confidential information that may arise under this CA.

2.4 Tasks for Participants. Each Participant will maintain regular and reasonable contact with the other Participant and engage in discussions regarding research collaboration and the research activities listed herein.

2.5 Funding. All commitments made in this CA are subject to the availability of appropriate funds and each party's budget priorities. Any endeavor involving reimbursement or contribution of funds between the parties to this CA will be handled in accordance with applicable laws, regulations, and procedures of the land and Institutes. Common resources can be availed through respective departments or universities.

ARTICLE III

(Duration, Termination and Amendment)

3.1 **Duration.** This CA shall remain in force for four (4) years from the date of the last signature. Either Participant may terminate this CA by providing 60 days advance written notice to the other Participant.

3.2 **Extension and Renewal.** The Participants may extend or renew this CA by agreement, confirmed in a written amendment signed by each Participant.

3.3 **Amendment.** No amendment of the terms of this CA will be effective unless made in writing and signed by each Participant.

ARTICLE I V

(General Matters)

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Dr.Gangadhara Somayaji K.S.
Registrar
Yenepoya(Deemed to be University)
University Road, Deralakatte,
Mangalore- 575 018, Karnataka

4.1 **Use of Names.** Except in promoting the activities proposed in Article 1.2 above among its faculty, staff, and students, neither Participant may use the name of the other Participant in any form of advertising or publicity without written permission. The Participants will seek written permission from one another by submitting the proposed use, well in advance of any deadline.

4.2 **Confidentiality.** In the course of the activities under this CA, it may be necessary for the Participants to disclose Confidential Information about the study. Unless otherwise expressly permitted in this agreement, any information, correspondence, financial statements, records, data, or information that is competitively sensitive and not generally known to the public, including formulations, analysis, inventions, improvements and activities of the disclosing Participant, disclosed by one Participant to the other Participant of this CA, and other documents shall be transmitted or communicated by either Participant to the other Participant that is marked as confidential or proprietary for this agreement ("Confidential Information") shall be received and treated in confidence, and shall not be used by the receiving Participant or disclosed by the receiving Participant without the prior written consent of the disclosing Participant, which consent shall not be unreasonably withheld or delayed. These restrictions on use or disclosure of information do not extend to any item of information which

(a) Is publically known at the time of the disclosure,

(b) Is lawfully received by the receiving Participant from a third party which does not have a confidential relationship to the disclosing Participant,

(c) The receiving Participant can demonstrate that it was in his possession or known the same before its receipt from the disclosing Participant, or

(d) The receiving Participant is required by law to disclose to government authorities (including courts).

Unless otherwise required under a subsequent binding agreement, each receiving Participant shall, at the expiry or termination of this agreement, return to the disclosing Participant any document provided by the disclosing Participant setting out as Confidential Information.

4.3 **Potential for Intellectual Property Development.** It is understood that activities contemplated under this CA are expected to be cooperative in nature and that Participating Researchers may collaborate in such research activities.

4.3.1 "Intellectual Property" or "IP" means all patentable discoveries, innovations, inventions, improvements, devices, equipment, and designs, conceived and reduced to practice under the term of and in the performance of this agreement.

4.3.2 Participants hereby agree that ownership of intellectual property rights generated as a result of the activities under this agreement will follow inventorship rules in accordance with applicable patent laws. Each Participant in this CA shall own the intellectual property (IP) conceived and first reduced to practice solely by the researchers in furtherance of projects or activities contemplated by this agreement. IP conceived or first reduced to practice jointly by the research team or agents of both parties shall be Jointly Owned in accordance

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Registrar
Yenepoya(Deemed to be University)
University Road, Deralakatte
Mangalore- 575 018, Karnataka

with applicable patent laws. "Jointly Owned" means either Participant may utilize jointly developed IP with mutual consent.

4.3.3 All copyrights, patents, trademarks, trade secrets, and any other intellectual property rights ("IPR") disclosed in connection with this CA shall solely remain the property of the Participant introducing and/or disclosing the same to the other Participant for this CA.

4.3.4 **Publication.** The parties agree that all journal articles, presentations and other communications created jointly by the parties from the activities conducted under the CA need to be reviewed and approved in accordance with the policies of both parties prior to publication or presentation. Substantial work carried out by either of the parties needs to be given due publication credit as an author.

(a) Appropriate acknowledgments must be given to the concerned departments/universities that have contributed to the research work pertaining to this CA in the form of resources.

(b) The author list and the order must be decided upon before initiating the projects based on the factual contributions. Any additions/deletions of the author(s) shall be decided after mutual agreement based on their contribution.

4.4 **Human and Animal Subjects in Research.** Participants agree that adequate safeguards shall be taken whenever using human or animal subjects in research, consistent with applicable laws and policies regarding the use of human and animal subjects, including training of such trainees, faculty, or staff who may be involved other than the participant. An institutional scientific review board/committee, Ethics Committee, and/or Animal Ethics committee approvals shall be sought in the respective institutions as applicable. Informed consent shall be obtained in accordance with national laws and regulations, international research standards, and accepted guidelines on good research practices and ethics. Each Participant shall, to the extent necessary for the legal conduct of activities under this CA, comply with the laws and regulations of the other Participant's Institution/ state/country.

4.6 **Results/ reporting and code of scientific integrity.** Both the parties hereby agree upon that the experiments performed and reported under this CA will be conducted with the highest level of transparency and scientific integrity. Any party if found to have indulged in scientific misconduct, such as data falsification, fabrication will be solely responsible for the misconduct and they shall not shift the blame on the other party. Any such misconduct shall be reported to the respective institution and proceedings will be conducted only by the institution/ organization to which the party indulging in scientific misconduct is affiliated.

4.7 **Notices.** The Participants must give all notices under this CA in writing. All communications must be sent to the addresses designated by the Participants.

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Dr.Gangadhara Somayaji K.S.
Registrar
Yenepoya(Deemed to be University)
University Road, Deralakatte
Mangalore- 575 018, Karnataka

4.8 **Arbitration and Dispute Redressal:** In case of any dispute between the parties to this Collaborative Agreement, the matter shall be referred to and be settled by arbitration. The arbitration shall be conducted by a single arbitrator to be appointed by the parties with their mutual consent. Arbitration proceedings shall be conducted in English as per the provisions of the Arbitration and Conciliation Act, 1996 and the rules framed thereon. Mangalore or Manipal shall be the places of arbitration.

4.10 **Non-Binding Nature.** This CA is not intended to and does not give any person who is not a Participant to it any rights to enforce any of its provisions. Nothing in this CA will be construed as creating a binding legal relationship between the Participants, with the exception of only Article IV herein which will survive the expiry or termination of this CA. This CA is a broad statement of intent which sets forth the general basis upon which the Participants wish to proceed. No legal liability will arise in respect of any subject matter hereof unless a subsequent binding agreement is negotiated, approved, executed and delivered by the Participants to this CA.

Dr. T. S. Keshava Prasad

Professor and Deputy Director
Center for Systems Biology and Molecular Medicine
Yenepoya Research Centre
Yenepoya (Deemed to be University)
Mangalore 575018 | India
Mobile: +91-9972250102
http://csbmm.yenepoya.edu.in/

Dr. Guruprasad Kalthur

Professor
Department of Clinical Embryology
Kasturba Medical College, Manipal
Manipal Academy of Higher Education
Manipal-576104, India

Forwarded by

The Registrar
Yenepoya (Deemed to be University)
Mangalore 575018 | India

Registrar
Yenepoya (Deemed to be University)
University Road, Deralakatte
Mangalore 575 018

Forwarded by:

Dr. Sharath K Rao
Dean
Kasturba Medical College, Manipal
Manipal Academy of Higher Education

The Dean
Kasturba Medical College, Manipal
Manipal Academy of Higher Education
Manipal- 576104, India

ATTESTED

Dr.Gangadhara Somayaji K.S.
Registrar
Yenepoya(Deemed to be University)
University Road, Deralakatte
Mangalore- 575 018, Karnataka

भारतीय आयुर्विज्ञान अनुसंधान परिषद
स्वास्थ्य अनुसंधान विभाग, स्वास्थ्य और परिवार
कल्याण मंत्रालय, भारत सरकार

Indian Council of Medical Research
Department of Health Research, Ministry of Health
and Family Welfare, Government of India

File No 5/10/FR/60/2020-RBMCH
Dated : 13-10-2021

The Registrar,
Yenepoya (Deemed to be University)
University Road, Deralakatte, Mangalore -575018

Subject:- Sanction of budget allotment for the Ad-hoc research proposal entitled "Understanding the clomiphene citrate-induced changes in secretory epithelial cells of human fallopian tube" under Dr. T S Keshava Prasad.

Sir/Madam,

The Director General, ICMR has been sanctioned the above-mentioned research proposal for the total period of 3 years with budget of **Rs. 38,60,198/- (Rupees Thirty Eight Lakhs Sixty Thousands and One Hundred Ninety Eight Only)** (copy enclosed). This proposal has been sanctioned initially for three year from **25-10-2021 to 24-10-2024** and it may be extended on the yearly basis after review of the work done during the period. The grant-in-aid will be given subject to the following condition.

1. The payment of the grant will be made in lump-sum to the Head of the Institute. The first installment of the grant will be paid generally as soon as report regarding appointment of the staff is received by the Council. The Staff appointed on the project should be paid as indicated in the budget statement.

2. The staff on the project will be recruited as per the rules and procedure of the host institute and second part of the undertaking be obtained from the employees of the project. The staff grant will not be released unless the required undertaking [part-II] from Head of the Institute is received in this office.

3. The demand for payment of the subsequent installment of the grant should be placed with the Council in the prescribed Performa.

4. Five copies of the annual progress report should be submitted to the ICMR every year after completion of ten months of the project giving complete actual details of the research work done. Failure to submit the report in time may lead to termination of project.

5. Subject to the condition that the grant will be utilize after following the provisions laid down in the GFRs-2017 & TA Rules.

6. Please keep the fund in the separate saving Bank Account opened for ICMR funded Research projects so that interest earned thereon is credited into the accounts.

7. The receipt of this letter may please be acknowledged.

Yours faithfully,

14/10/2021

(Ramesh Kumar)
Administrative Officer
For Director General

Copy together with a copy of the budget statement forwarded to information to

1. **Dr. T S Keshava Prasad**, Professor and Deputy Director Center for system biology and Molecular Medicine, Yenepoya Research Center, Yenepoya (Deemed to be University), Mangalore
2. Accounts. V. for information.
3. IRIS Cell No. 2020-3410
4. Sh. Rohitash Singh, PTO, Division of RBMCH, ICMR

ATTESTED

Dr.Gangadhara Somayaji K.S.
Registrar
Yenepoya(Deemed to be University)
University Road, Deralakatte
Mangalore- 575 018, Karnataka

5/10/FR/60/2020-RBMCH
E-office - 111444
Date of Start : 25-10-2021
Duration: 3 years
IRIS Cell No. 2020-3410
RFC No. RBMCH/Adhoc/28/2021-22 dated : 07/10/2021

Project entitled **"Understanding the clomiphene citrate-induced changes in secretory epithelial cells of human fallopian tube" under Dr. T S Keshava Prasad.**

Budget for 1st year
25-10-2021 to 24-10-2022

Particular	1st year
Man Power	
Senior Research Fellow (SRF) One Rs. 35000/- x 16% HRA = 40,600 x 12 =	487200
Non-Recurring	
Proteome Discover Software upgradation	400000
Consumable 3kDa filters, Trypsin, HPLC columns, spectrometry, Other Plastic ware, consumables & chemicals	1000000
Contingency	25000
Travel	25000
Overhead 3% Institutional Charges	45,366
Total	19,82,566

Total Budget allotment of Rs. 19,82,566/- (Rupees Nine Lakh Twenty Three Thousand and Sixty Three Only)

14/10/2021
Administrative Officer
For Director General

ATTESTED
Dr.Gangadhara Somayaji K.S.
Registrar
Yenepoya(Deemed to be University)
University Road, Deralakatte
Mangalore- 575 018, Karnataka

FORMAT OF RESEARCH PLAN

1. **Title of the proposed research project:** Understanding the clomiphene citrate-induced changes in secretory epithelial cells of human fallopian tube

2. **Summary (up to 250 words):**

Background: Clomiphene citrate (CC) is the most preferred first line drug for infertility treatment involving mild ovulation induction. However, there are substantial reports in the literature suggesting that though it is a good ovulation induction drug, the pregnancy rate is much lower compared to other drugs like letrozole. In spite of lack of understanding on these aspects, it is still being used widely.

Novelty: In the present study we propose to use secretory epithelial cells grown from fallopian tubes of healthy human undergoing tubectomy to understand the functional changes including the secretome profile which is not studied so far.

- Objectives: a) To understand the effect of clomiphene citrate on the function of human fallopian tube secretory epithelial cells in vitro; b) To elucidate the response of human fallopian tube secretory cells towards human spermatozoa following exposure to CC; c) To analyze CC-induced variation in secretome profile of human fallopian tube secretory cells at both proteome and metabolome levels and to assess the influence of secretome on the sperm function under vitro conditions

Methods: Secretory epithelial cells from human fallopian tube will be collected and cultured in vitro. The cells will be treated with various concentrations of CC to understand the genetic and functional changes in the secretory cells. The secremote analysis at both proteomic and metabolomics level will be performed using mass spectrometric technique. Further, the changes in the interaction with spermatozoa will be assessed by co-culturing the human spermatozoa with secretory cells exposed to clomiphene citrate. The in vitro findings will be validating by in vivo experiments using mouse model.

ATTESTED

Dr Gangadhara Samaya K.S.
Registrar
Yenepoya(Deemed to be University)
University Road, Deralakatte
Mangalore- 575 018, Karnataka

DECLARATION AND ATTESTATION

i. I/We have read the terms and conditions for ICMR Research Grant. All necessary institutional facilities will be provided if the research project is approved for financial assistance.

ii. I/We agree to submit within one month from the date of termination of the project the final report and a list of articles, both expendable and non-expendable, left on the closure of the project.

iii. I/We agree to submit audited statement of accounts duly audited by the auditors as stipulated by the ICMR.

iv. It is certified that the equipment(s) is/are not available in the Institute/Department or these are available but cannot be spared for the project

v. It is further certified that the equipment(s) required for the project have not been purchased from the funds provided by ICMR for another project(s) in the Institute.

vi. I/We agree to submit (online) all the raw data (along with descriptions) generated from the project to the ICMR Data Repository within one month from the date of completion /termination of the project.

If any equipment already exists with the Department/Institute, the investigator should justify purchase of the another equipment

Signature of the:

a) Principal Investigator; Dr Guruprasad Kalthur

b) Co- PI I Dr Keshav Prasad

Co-investigators (3)

1. Dr Pratap Kumar
2. Dr Satish Kumar Adiga
3. Dr Shamprasad Varija Raghu

c) Head of the Department

Signature of the Head of the Institution with seal

Dr. Shrinath K Rao
Dean
Kasturba Medical College, Manipal
Manipal Academy of Higher Education

9/1/2020

ATTESTED

Dr.Gangadhara Somayaji K.S.
Registrar
Yenepoya(Deemed to be University)
University Road, Deralakatte
Mangalore- 575 018, Karnataka

Dr. Meera Jacob

From

02/11/2020

Manipal

Dr. Vrinda Hari Ankolekar (Principal Investigator)
Associate Professor of Anatomy
Kasturba Medical College, Manipal
MAHE, Manipal, India

To,

H.O.D, Anatomy department
Yenepoya Medical College
Mangaluru

Dear Sir,

Sub: Permission to conduct collaborative research work

As a part of our anthropometry project entitled 'Estimation of sexual dimorphism using adult human mandibles of South Indian Origin- A multicentric study' (IEC-476/2019), we intend to procure data from 150 adult human mandibles.

This project's intended outcomes are to provide a South Indian database of mandibles to estimate sexual dimorphism through metric & nonmetric parameters (preferably a published version in a high-impact journal).

In this regard, we request your permission to procure the mandibles for data collection from the Department of Anatomy of Yenepoya Medical College. We have discussed this with one of your Anatomy faculty Dr. Meera Jacob and is willing to contribute. We assure the credit for the contribution in the future publication of this research. Therefore, it would be of great help to assign Dr. Meera Jacob to collaborate with us for this research work.

We have taken permission from the Dean, Kasturba Medical College, Manipal, and the letter is attached with this.

Thank you,
Sincerely
Vrinda A
Dr Vrinda Hari Ankolekar

Permitted.
4/11/2020
Signature of the HOD
Professor & Head
Dept. of Anatomy
Yenepoya Medical College
Mangalore-575018

ATTESTED
Dr.Gangadhara Somayaji K.S.
Registrar
Yenepoya(Deemed to be University)
University Road, Deralakatte
Mangalore- 575 018, Karnataka

Cancer Biomarkers 31 (2021) 361–373
DOI 10.3233/CBM-203077
IOS Press

Molecular alterations in oral cancer between tobacco chewers and smokers using serum proteomics

Varshasnata Mohanty[a,1], Yashwanth Subbannayya[a,b,1], Shankargouda Patil[c], Riaz Abdulla[d], Mandakulutur S. Ganesh[e], Arnab Pal[f], Jay Gopal Ray[g,k], David Sidransky[h], Harsha Gowda[a,i,j], T.S. Keshava Prasad[a,*] and Aditi Chatterjee[a,i,j,*]

[a]*Center for Systems Biology and Molecular Medicine, Yenepoya Research Centre, Yenepoya (Deemed to be University), Mangalore, Karnataka, India*

[b]*Centre of Molecular Inflammation Research (CEMIR), and Department of Clinical and Molecular Medicine (IKOM), Norwegian University of Science and Technology, Trondheim, Norway*

[c]*Division of Oral Pathology, College of Dentistry, Department of Maxillofacial Surgery and Diagnostic Sciences, Jazan University, Jazan, Saudi Arabia*

[d]*Department of Oral Pathology, Yenepoya Dental College, Yenepoya (Deemed to be University), Mangalore, Karnataka, India*

[e]*Department of Surgical Oncology, Vydehi Institute of Oncology, Bangalore, Karnataka, India*

[f]*Department of Biochemistry, Postgraduate Institute of Medical Education and Research, Chandigarh, India*

[g]*Department of Oral Pathology, Dr. R Ahmed Dental College and Hospital, Kolkata, West Bengal, India*

[h]*Department of Otolaryngology-Head and Neck Surgery, Johns Hopkins University, School of Medicine, Baltimore, MD, USA*

[i]*Institute of Bioinformatics, International Tech Park, Bangalore, Karnataka, India*

[j]*Manipal Academy of Higher Education, Manipal, Karnataka, India*

[k]*Department of Pathology, Burdwan Dental College and Hospital, Burdwan, West Bengal, India*

Received 3 November 2020
Accepted 14 April 2021

Abstract.

BACKGROUND: Tobacco exposure (through smoking or chewing) is one of the predominant risk factors associated with the development of oral squamous cell carcinoma (OSCC). Despite the growing number of patients diagnosed with OSCC, there are few circulating biomarkers for identifying individuals at a higher risk of developing the disease. Successful identification of candidate molecular markers for risk assessment could aid in the early detection of oral lesions and potentially be used for community screening of high-risk populations.

OBJECTIVE: Identification of differentially expressed proteins in the serum of oral cancer patients which can serve as biomarkers for the diagnosis of the onset of oral cancer among tobacco users.

METHODS: We employed a tandem mass tag (TMT)-based quantitative proteomics approach to study alterations in the serum proteomes of OSCC patients based on their tobacco exposure habits (chewing and smoking) compared to healthy individuals with no history of using any form of tobacco or any symptoms of the disease.

[1]These authors contributed equally to the manuscript.

*Corresponding authors: T.S. Keshava Prasad and Aditi Chatterjee, Center for Systems Biology and Molecular Medicine, Yenepoya (Deemed to be University), Mangalore 575018, India. Tel.: +91 9972250102 and +91 9591100113; E-mail: keshav@yenepoya.edu.in and aditixchatterjee@gmail.com.

Manipal.

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Early detection of leptospirosis using Anti-LipL32 carbon nanotube immunofluorescence probe

Kannan Sapna,[1,2] Mohammed Tarique,[2] Ashaiba Asiamma,[2] Terikere Nagaraj Ravi Kumar,[4] Vishwanath Shashidhar,[4] Ananthapadmanabha Bhagwath Arun,[2] and Kariate Sudhakara Prasad[1,3,*]

Nanomaterial Research Laboratory (NMRL), Nano Division, Yenepoya Research Centre, Yenepoya (Deemed to Be University), Deralakatte, Mangalore 575 018, India,[1] Yenepoya Research Centre, Yenepoya (Deemed to Be University), Deralakatte, Mangalore 575 018, India,[2] Centre for Nutrition Studies, Yenepoya (Deemed to Be University), Deralakatte, Mangalore 575 018, India,[3] and Department of Microbiology, Kasturba Medical College, Manipal Academy of Higher Education, Manipal 576 104, India[4]

Received 3 February 2020; accepted 2 June 2020
Available online xxx

Leptospirosis is a widespread zoonosis and an emerging public health problem. Leptospirosis symptoms are often confused or misdiagnosed with other febrile illness like malaria, viral hepatitis, influenza, dengue, typhoid, melioidosis, and scrub typhus as the clinical manifestations are almost similar. Therefore, early and accurate diagnosis of leptospirosis is indeed critical for proper and prompt treatment. Herein, we report the development of single-walled carbon nanotubes based immunofluorescence probe (Carbo-Lip) for the detection of leptospirosis at an early phase by utilising major outer membrane protein, LipL32 of *Leptospira*. The Carbo-Lip probe was fabricated through immuno recognition method with fluorescent dye functionalized LipL32 monoclonal antibodies (mAbs), secondary antibody and *Leptospira*. Surface characterization studies such as Fourier transform infrared spectroscopy with the attenuated total reflectance, scanning electron microscopy, transmission electron microscopy, Zeta potential, and X-ray photoelectron spectroscopy techniques were used to demonstrate the successful fabrication of Carbo-Lip probe. The sensor probe was capable of detecting the presence of leptospires at a lower concentration of 10^3/ml, and could detect 10^2 leptospires in 100 µL of sample within 3 h of the test conditions, and was stable up to 2 weeks. This Carbo-Lip probe was further tested and validated for its capacity to detect *Leptospira* in clinical samples, which exhibited high selectivity and specificity towards *Leptospira* even in the presence of malaria and dengue. Our results were consistent with microscopic agglutination test, which is known as gold standard, immunoglobulin M (IgM) enzyme-linked immunoassay (ELISA), IgM spot test, and culture tests for the diagnosis of *Leptospira* infection.

[**Key words:** Leptospirosis; Diagnosis; Carbon nanotubes; Immunofluorescence sensor; LipL32]

Leptospirosis is life-threatening zoonosis mostly affecting people in the developing countries (1), and is commonly prevalent in the tropical and subtropical regions. Its global morbidity and mortality were recently estimated to 1.03 million cases and 58,900 deaths annually, mostly in resource-poor settings (2). The major outbreaks of leptospirosis occur during flooding and adventure tourism (1,3,4). The disease is associated with multi-organ damage/failures with high mortality rate and is often confused with other febrile illness such as dengue, malaria, and scrub typhus that are frequently reported from the endemic areas (5–7). Moreover, co-infections with other febrile infections have been reported, which also leads to misdiagnosis of leptospirosis (8–10).

At present techniques such as culture, microscopic agglutination test (MAT), immunoglobulin M (IgM) enzyme-linked immunoassay (ELISA), dark field microscopy, conventional polymerase chain reaction (PCR), real time PCR and high resolution melting analysis were employed for diagnosis of leptospirosis in clinical laboratories, nevertheless aforementioned techniques have limitations (1,11–14). One of the most common methods such as culturing of *Leptospira* is time-consuming, since it takes several weeks to months for proper growth. The gold standard for diagnosis of leptospirosis is MAT, which is time consuming, requires live serovars, skilled personnel and is cumbersome. Notably, sensitivity of MAT is high (100%) only after the fourth week of infection. Current practice of using IgM ELISA test at local hospitals lacks sensitivity, and gives false positive results. On the other hand, dark field microscopy lacks specificity and sensitivity, requires at least good amount of leptospires/mL in the sample of interest (11). PCR is expensive, besides requires sophisticated instrumentations, and sample processing. This technique is not useful in strain identifications (1,12). Hence, diagnosis of leptospirosis is a major challenge. Moreover, early and accurate diagnosis of leptospirosis is critical for proper and prompt treatment, which is life-saving for patients with severe illness.

By utilizing major outer membrane protein of pathogenic *Leptospira*, LipL32 (5,15–17), quite a few attempts have been reported for the detection of leptospirosis, such as the electrochemical sensor (18), and immunochromatographic assays (19). Notably, immunoassay using leptospiral lipopolysccaride (20,21), dot ELISA (16), anti-*Leptospira*/gold nanoparticles based colorimetric assay

* Corresponding author at: Nanomaterial Research Laboratory (NMRL), Nano Division, Yenepoya Research Centre, Yenepoya (Deemed to Be University), Deralakatte, Mangalore 575 018, India. Tel.: +91 824 220 4668 Ext. 2035; fax: +91 824 220 4667.
E-mail address: ksprasadnair@yenepoya.edu.in (K.S. Prasad).

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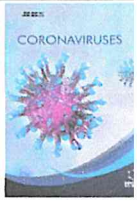
RESEARCH ARTICLE



High Mutation Rate Leads to Fitness Loss for Coronavirus Quasispecies

Khalid Parvez[1,*], Arun A. Bhagwath[1], Rekha P.D.[1], Sharif Hamed R. Almutiri[2], Ziyad Al Zeyadi[2] and Suman V. Budihal[3]

[1]*Yeneoya Research Centre, Yenepoya (Deemed to be University), Deralakatte, Mangalore, Karnataka, India;* [2]*College of Applied Medical Science, Department of Clinical Laboratory Science, Dawadmi, Shaqra University, Saudi Arabia;* [3]*Department of Physiology, Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Manipal, Karnataka, India*

Abstract: ***Background*:** RNA viruses evolve very fast, with a mutation rate of 10^3 to 10^5 base substitution per nucleotides per copy. The mutation is a survival strategy for the viruses, which leads them to survive in the new host. Fitness is defined as the replication capacity of the virus in an experimental setup. Generally, the large population passage of the virus leads to fitness gain, but the world data of the coronavirus infection and death shows the flattened curve with time. It is contradictory to the principle of fitness gain due to large population passage. The coronavirus is losing its potency but remains infectious as it is passaging into millions that leads to a decline in the death of COVID patients and high recovery rates. Fitness loss of coronaviruses attributed to a high level of mutation in the RNA genome as well as host immune response. The current outbreak of SARS CoV-2 is surfaced in December 2019 in Hubei province of China and considered as bats/pangolin origin, spreading 235 countries of the world, infecting nearly 31,664,104 people, and claimed nearly 972,221 lives as of September 24, 2020 (Death rate approximately 3%). This coronavirus has passaged into 31,664,104 people from the beginning of this pandemic until September 24, 2020. Now the virus is losing potency rather than being monotonous and continuous in producing virus-related complications. The population is still getting infected at the same rate, but the severity of the disease is reduced due to the potency of the virus diminished due to the passage effect as well as fitness loss of the virus due to high mutation rates. The death rate is reduced to 3% as compared to 6% in June 2020, when this paper was first submitted.

***Objective*:** The purpose of the study is to prove the fact that the coronavirus loses its potency with time but, they remain infective. It becomes more infectious due to mutation of the gene but loses the capacity to kill the host.

***Methods*:** Since the WHO announces the COVID-19 outbreak is an emergency of international concern, every country in the world is taking many measures to mitigate the viral load to their population. Simultaneously, the WHO, CDC USA, CDC Europe, and much other organization is updating the COVID cases and death online daily as reported by the respective country. With the help of the COVID-19 outbreak data published by the European CDC and ourworldindata.org, we correlate the total cases of coronavirus and total death in the top ten affected countries in the world. We also link the trends of total cases *vs.* total death and total new cases *vs.* total new death related to COVID-19 in Germany, Spain, the United Kingdom, Italy, and New Zealand from January 30, 2020, until September 24, 2020. The reason to select these countries for the study is that these countries updating the COVID cases and deaths regularly and said to achieve the peak of COVID related infections and recovering from the pandemic.

***Results*:** We have tried to correlate the high mutation rate of the virus that leads to losing its potency to severe infection and death in the human. Viral extinction through high mutation could be considered as the new anti-viral strategies.

***Conclusion*:** Coronavirus is losing its potency to causing death to the human. The new infection is still being reported from every corner of the world, but the death rate is significantly decreasing.

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* Address correspondence to this author at the Yeneoya Research Centre, Yenepoya (Deemed to be University), Deralakatte, Mangalore, Karnataka, India; Tel: +91-9900412430; E-mail: drkhalidpm@gmail.com

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NUTS AND BOLTS

Molecular alterations in oral cancer using high-throughput proteomic analysis of formalin-fixed paraffin-embedded tissue

Varshasnata Mohanty[1] · Yashwanth Subbannayya[1,2] · Shankargouda Patil[3] · Vinuth N. Puttamallesh[4] · Mohd. Altaf Najar[1] · Keshava K. Datta[1] · Sneha M. Pinto[1,2] · Sameera Begum[5] · Neeta Mohanty[6] · Samapika Routray[6,7] · Riaz Abdulla[5] · Jay Gopal Ray[8,9] · David Sidransky[10] · Harsha Gowda[1,4,11] · T. S. Keshava Prasad[1] · Aditi Chatterjee[1,4,11]

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Abstract

Loss of cell differentiation is a hallmark for the progression of oral squamous cell carcinoma (OSCC). Archival Formalin-Fixed Paraffin-Embedded (FFPE) tissues constitute a valuable resource for studying the differentiation of OSCC and can offer valuable insights into the process of tumor progression. In the current study, we performed LC–MS/MS-based quantitative proteomics of FFPE specimens from pathologically-confirmed well-differentiated, moderately-differentiated, and poorly-differentiated OSCC cases. The data were analyzed in four technical replicates, resulting in the identification of 2376 proteins. Of these, 141 and 109 were differentially expressed in moderately-differentiated and poorly differentiated OSCC cases, respectively, compared to well-differentiated OSCC. The data revealed significant metabolic reprogramming with respect to lipid metabolism and glycolysis with proteins belonging to both these processes downregulated in moderately-differentiated OSCC when compared to well-differentiated OSCC. Signaling pathway analysis indicated the alteration of extracellular matrix organization, muscle contraction, and glucose metabolism pathways across tumor grades. The extracellular matrix organization pathway was upregulated in moderately-differentiated OSCC and downregulated in poorly differentiated OSCC, compared to well-differentiated OSCC. PADI4, an epigenetic enzyme transcriptional regulator, and its transcriptional target HIST1H1B were both found to be upregulated in moderately differentiated and poorly differentiated OSCC, indicating epigenetic events underlying tumor differentiation. In conclusion, the findings support the advantage of using high-resolution mass spectrometry-based FFPE archival blocks for clinical and translational research. The candidate signaling pathways identified in the study could be used to develop potential therapeutic targets for OSCC.

Keywords Cancer pathology · Pressure cycling technology · Molecular medicine · Cancer grade · Quantitative proteomics · Tumor differentiation

Abbreviations

DTT	Dithiothreitol
FDR	False Discovery Rate
FFPE	Formalin-Fixed Paraffin Embedded
GO	Gene Ontology
IAA	Iodoacetamide
MS/MS	Tandem Mass Spectrometry
OSCC	Oral Squamous Cell Carcinoma
PCT	Pressure Cycling Technology
PSM	Peptide Spectrum Match
SDS	Sodium Dodecyl Sulfate
TEABC	Triethyl Ammonium Bicarbonate
TMT	Tandem Mass Tags

Introduction

Cancers of the lip and oral cavity arise primarily from epithelial cells, and 90% of these are composed of oral squamous cell carcinoma (OSCC) by origin (Miranda-Filho and Bray 2020). OSCC arises in various anatomical locations

Varshasnata Mohanty and Yashwanth Subbannayya contributed equally to the manuscript.

✉ T. S. Keshava Prasad
keshav@yenepoya.edu.in

✉ Aditi Chatterjee
aditixchatterjee@gmail.com

Extended author information available on the last page of the article

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NUTS AND BOLTS

An assembly of galanin–galanin receptor signaling network

Lathika Gopalakrishnan[1,2,3] · Oishi Chatterjee[1,3,4] · Chinmayi Raj[5] · Deepshika Pullimamidi[5] · Jayshree Advani[1,8] · Anita Mahadevan[6,7] · T. S. Keshava Prasad[3]

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Abstract

The galanin receptor family of proteins is present throughout the central nervous system and endocrine system. It comprises of three subtypes—GalR1, GalR2, and GalR3; all of which are G-protein-coupled receptors. Galanin predominantly acts as an inhibitory, hyper-polarizing neuromodulator, which has several physiological as well as pathological functions. Galanin has a role in mediating food intake, memory, sexual behavior, nociception and is also associated with diseases such as Alzheimer's disease, epilepsy, diabetes mellitus, and chronic pain. However, the understanding of signaling mechanisms of the galanin family of neuropeptides is limited and an organized pathway map is not yet available. Therefore, a detailed literature mining of the publicly available articles pertaining to the galanin receptor was followed by manual curation of the reactions and their integration into a map. This resulted in the cataloging of molecular reactions involving 64 molecules into five categories such as molecular association, activation/inhibition, catalysis, transport, and gene regulation. For enabling easy access of biomedical researchers, the galanin–galanin receptor signaling pathway data was uploaded to WikiPathways (https://www.wikipathways.org/index.php/Pathway:WP4970), a freely available database of biological pathways.

Keywords Galaninergic neuromodulatory system · NetPath · Post-translational modifications · Protein–protein interactions · Neuromodulation

Abbreviations

GAL	Galanin
GalR	Galanin receptor
GALP	Galanin-like peptide
CNS	Central nervous system
GPCR	G-protein coupled receptor
CREB	cAMP response element-binding protein
PKA	Protein kinase A
MAPK	Mitogen activated protein kinase

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✉ T. S. Keshava Prasad
keshav@yenepoya.edu.in

Lathika Gopalakrishnan
lathika@ibioinformatics.org

Oishi Chatterjee
oishi@ibioinformatics.org

Chinmayi Raj
chinmayigraj@gmail.com

Deepshika Pullimamidi
deepshikag.puli@gmail.com

Jayshree Advani
jayshree.ibioinformatics@gmail.com

Anita Mahadevan
mahadevananita@gmail.com

[1] Institute of Bioinformatics, International Tech Park, Bangalore 560066, India

[2] Manipal Academy of Higher Education (MAHE), Manipal 576104, India

[3] Center for Systems Biology and Molecular Medicine, Yenepoya Research Centre, Yenepoya (Deemed to be University), Mangalore 575018, India

[4] Amrita School of Biotechnology, Amrita Vishwa Vidyapeetham, Kollam 690525, India

[5] School of Life Sciences, Pondicherry University, Puducherry 605014, India

[6] Department of Neuropathology, National Institute of Mental Health and Neuro Sciences, Bangalore, India

[7] Human Brain Tissue Repository, National Institute of Mental Health and Neuro Sciences, Neurobiology Research Centre, Bangalore, India

[8] Present Address: Neurobiology-Neurodegenration and Repair Laboratory, National Eye Institute, National Institute of Health, Bethesda, MD 20982, USA

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Digging Deeper for the Eye Proteome in Vitreous Substructures: A High-Resolution Proteome Map of the Normal Human Vitreous Base

Varshasnata Mohanty,[1,*] Sneha M. Pinto,[1,*] Yashwanth Subbannayya,[1] Mohd. Altaf Najar,[1] Kalpana Babu Murthy,[2,3] Thottethodi Subrahmanya Keshava Prasad,[1] and Krishna R. Murthy[2–5]

Abstract

Mapping the normal eye proteome in healthy persons is essential to unravel the molecular basis of diseases impacting visual health. The vitreous occupies a large portion of the human eye between the lens and the retina and plays a significant role in vitreoretinal diseases as well as maintaining clarity in the visual field, providing nutrition to the lens, and protecting the eye from mechanical shocks. It comprises four distinct anatomical regions, namely the vitreous core, vitreous cortex, vitreous base, and anterior hyaloid. Among these, the vitreous is attached to other substructures in the eye by the vitreous base, which is its strongest point of attachment. Alterations in vitreous substructures have been reported in several vitreoretinal disorders, including vitreomacular traction, vitreoretinopathies, and age-related macular degeneration. There has been limited knowledge on proteomics variations at a resolution of vitreous substructures, including the functionally and pathophysiologically significant vitreous base. We report here new findings on the proteome map of the vitreous base in normal healthy tissue. We employed a global, unbiased proteomic profiling approach resulting in the identification of 6511 proteins. Of these, 302 proteins were involved in metabolic processes essential for energy utilization. Moreover, we identified several structural and nutrient transport proteins. Notably, the identified proteome repertoire indicates that the vitreous base might possess additional physiological functions and may not be a passive structure. This study constitutes the most extensive catalog of vitreous base proteins to our knowledge and offers novel insights as a baseline for future studies on the pathobiology of various eye diseases. These data also invite us to consider a potentially more active functional role for the vitreous base in eye physiology and visual health.

Keywords: eye proteome, visual health, omics technology, ophthalmology, LC-MS/MS, protein network

Introduction

THE VITREOUS IS ONE OF THE LARGEST STRUCTURES in the eye and lies adjacent to the retina posteriorly and the lens and ciliary body anteriorly. The human vitreous comprises four distinct anatomical regions, namely the vitreous core, vitreous cortex, vitreous base, and anterior hyaloid. The vitreous core is surrounded by the cortical vitreous, which comprises the anterior hyaloid membrane and the vitreous base. It is the largest compartment and houses the vitreous humor. The anterior hyaloid membrane is made up of condensation of protein fibers and is attached to the posterior lens. The vitreous base consists of dense collagen fibers, which are implanted in an area that extends 2 mm anteriorly to adjoining pars plana region of the ciliary body and 3 mm posteriorly to peripheral retina posterior to the *ora serrata*. The fibers also extend radially in toward the vitreous gel. The vitreous base is the strongest point of attachment of the vitreous.

[1]Center for Systems Biology and Molecular Medicine, Yenepoya Research Centre, Yenepoya (Deemed to be University), Mangalore, India.
[2]Department of vitreo retina, Vittala International Institute of Ophthalmology, Bangalore, India.
[3]Department of vitreo retina, Prabha Eye Clinic and Research Centre, Bangalore, India.
[4]Institute of Bioinformatics, International Technology Park, Bangalore, India.
[5]Manipal Academy of Higher Education, Manipal, India.
*These authors contributed equally to this work.

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Research Article

Intracranial Aneurysm Biomarker Candidates Identified by a Proteome-Wide Study

Tanavi Sharma,[1,*] Keshava K. Datta,[2,*] Munish Kumar,[1] Gourav Dey,[2] Aafaque Ahmad Khan,[2] Kiran Kumar Mangalaparthi,[2] Poonam Saharan,[1] Shobia Chinnapparaj,[1] Ashish Aggarwal,[3] Navneet Singla,[3] Sujata Ghosh,[4] Amit Rawat,[5] Sivashanmugam Dhandapani,[3] Pravin Salunke,[3] Rajesh Chhabra,[3] Dalbir Singh,[6] Aastha Takkar,[7] Sunil K. Gupta,[3] Thottethodi Subrahmanya Keshava Prasad,[2,8] Harsha Gowda,[2,8,9] Kanchan K. Mukherjee,[3,†] Akhilesh Pandey,[2,9,10,11] and Hemant Bhagat[1]

Abstract

The scientific basis of intracranial aneurysm (IA) formation, its rupture and further development of cerebral vasospasm is incompletely understood. Aberrant protein expression may drive structural alterations of vasculature found in IA. Deciphering the molecular mechanisms underlying these events will lead to identification of early detection biomarkers and in turn, improved treatment outcomes. To unravel differential protein expression in three clinical subgroups of IA patients: (1) unruptured aneurysm, (2) ruptured aneurysm without vasospasm, (3) ruptured aneurysm who developed vasospasm, we performed untargeted quantitative proteomic analysis of aneurysm tissue and serum samples from three subgroups of IA patients and control subjects. Candidate molecules were then validated in a larger cohort of patients using enzyme-linked immunosorbent assay. A total of 937 and 294 proteins were identified from aneurysm tissue and serum samples, respectively. Several proteins that are known to maintain structural integrity of vasculature were found to be dysregulated in the context of aneurysm. *ORM1*, a glycoprotein, was significantly upregulated in both tissue and serum samples of unruptured aneurysm patients. We employed a larger cohort of subjects ($n=26$) and validated *ORM1* as a potential biomarker for screening of unruptured aneurysms. Samples from ruptured aneurysms with vasospasm showed significant upregulation of *MMP9*, a protease, compared with ruptured aneurysms without vasospasm. We validated *MMP9* as a potential biomarker for vasospasm in a larger cohort ($n=52$). This study reports the first global proteomic analysis of the entire clinical spectrum of IA. Furthermore, this study suggests *ORM1* and *MMP9* as potential biomarkers for unruptured aneurysm and cerebral vasospasm, respectively.

Keywords: cerebral vasospasm, intracranial aneurysm, mass spectrometry, proteomics, subarachnoid hemorrhage, biomarker

[1]Division of Neuroanesthesia, Department of Anesthesia and Intensive Care, Post Graduate Institute of Medical Education and Research, Chandigarh, India.
[2]Institute of Bioinformatics, International Tech Park, Bangalore, India.
Departments of [3]Neurosurgery and [4]Experimental Medicine and Biotechnology, Post Graduate Institute of Medical Education and Research, Chandigarh, India.
[5]Pediatric Allergy and Immunology Unit, Advanced Pediatrics Centre, Post Graduate Institute of Medical Education and Research, Chandigarh, India.
Departments of [6]Forensic Medicine and [7]Neurology, Post Graduate Institute of Medical Education and Research, Chandigarh, India.
[8]Center for Systems Biology and Molecular Medicine, Yenepoya Research Centre, Mangalore, India.
[9]Manipal Academy of Higher Education, Manipal, India.
[10]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA.
[11]Center for Individualized Medicine, Mayo Clinic, Rochester, Minnesota, USA.
*These two authors contributed equally to this work.
†Deceased.