UNIVERSITY OF MYSORE

YUVARAJA'S COLLEGE

(A constituent Autonomous College of the University of Mysore)
(Re-Accredited "B^{++}" Grade by NAAC)
JLB Road, Mysore-570005

Department Of Molecular Biology

ALUMNI INTERACTION SESSION #4

Title: DNA Methylation regulates MEG3 and miR-379/656 cluster regulation in Hepatocellular Carcinoma

Shreyas Hulusemane Karunakara

Lady Tata Junior Research Fellow| Liver Research Laboratory|
Centre of Excellence in Molecular Biology and Regenerative Medicine(ICMR—CcoE) |
JSS Medical College and Hospital
Integrated Masters in Molecular Biology |University of Mysore| Jul 2016 - Oct 2021`

01st JANUARY 2025, 10 AM
Lecture hall, Department of Molecular Biology

DNA&S
DEPARTMENT'S NETWORK OF ALUMNI & STUDENTS

Report on DNA Methylation regulates MEG3 and miR-379/625 cluster regulation in Hepatocellular Carcinoma (HCC)

Introduction

The report summarizes the interaction session delivered by Shreyas H K on the topic of DNA methylation and its role in regulating MEG3 (Maternally Expressed Gene 3) and the miR-379/625 cluster in Hepatocellular carcinoma (HCC). The session explored how these epigenetic modifications contribute to the progression of HCC and the potential for novel Biomarker interventions targeting DNA methylation.

Session details

Date and time: - 01/01/2025 and 10:00 AM

Duration: - lasting for 80mins

Location: - Lecture hall, Department of Molecular Biology.

Mr. Shreyas Hulusemane Karunakara is working as a Lady Tata Junior Research Fellow at the Liver Research Laboratory, Centre of Excellence in Molecular Biology and Regenerative Medicine (ICMR-CcoE) at JSS Medical College and Hospital. He completed his Integrated Master's in Molecular Biology from the University of Mysore between July 2016 and October 2021. He is primarily interested in the epigenetics triad, which includes DNA methylation, histone modifications, and regulatory RNAs. He briefly introduces the importance of DNA methylation, MEG3, and the miR-379/625 cluster in the context of Hepatocellular Carcinoma (HCC).

Discussion and Research findings

The liver is a vital organ responsible for detoxification, protein synthesis, and production of biochemicals necessary for digestion, such as bile. It also stores glycogen, regulates blood clotting, and metabolizes fats, proteins, and carbohydrates. Additionally, it plays a key role in immune function and hormone regulation. Hepatocellular carcinoma (HCC) is a type of liver cancer that originates in hepatocytes, the main cells of the liver. It is commonly associated

with chronic liver diseases such as cirrhosis, often caused by hepatitis B, C infections or Wilson disease. Hepatocellular carcinoma (HCC) develops in the parenchymal cells of the liver, driven by genetic mutations such as aneuploidy, single nucleotide polymorphisms (SNPs), and recessive mutations. These mutations lead to clonal expansion of hepatocytes, which accumulate over time and contribute to the dysregulation of oncogenes and tumor suppressor genes. For example, the tumor suppressor gene **p53**, when mutated or inactivated, can no longer effectively regulate cell cycle arrest and apoptosis, allowing abnormal hepatocytes to proliferate and replace normal liver cells, ultimately leading to HCC. These alterations in tumor suppressor genes, oncogenes, SNPs, and recessive mutations serve as critical biomarkers for the diagnosis and progression of HCC, offering potential targets for early detection and therapeutic intervention. He is particularly interested in how specific non-coding RNA networks are influenced by DNA methylation in the context of Hepatocellular Carcinoma (HCC). DNA methylation regulates gene expression by adding a methyl group to cytosine residues in CpG islands, typically within gene promoter regions. This modification can silence gene expression by preventing transcription factor binding, whereas reduced methylation can activate gene expression.

He focused on a specific region at locus 32.2p arm of chromosome 14, which harbors a cluster of microRNAs (miR-379 to miR-656). His research involved studying the role of these microRNAs in HCC by examining the MEG3 gene and its promoter. MEG3, a known tumor suppressor gene, is downregulated in cancer patients compared to healthy individuals, as shown by Nanostring analysis in HEPG2 and HUH7 cell lines. The microRNAs from the cluster were ligated to an adapter and tagged with fluorescent probes. Fluorescent microscopy results indicated that the emission counts were proportional to the levels of specific microRNAs, revealing that five microRNAs from this cluster were downregulated in HCC, contributing to tumor progression.

To further investigate the role of MEG3 in HCC, he cloned the putative promoter region of MEG3 into a 1.5kb pGL3 Basic expression vector using molecular cloning techniques. Diagnostic digestion confirmed successful cloning and correct orientation of the promoter region. To assess promoter activity, plasmids were transfected into HEPG2 cells using a lipofectamine-mediated gene transfer method. Dual luciferase assays with Renilla and firefly luciferase reporters showed that the putative promoter exhibited activity compared to the pGL3 Basic vector, confirming its functional role in gene expression.

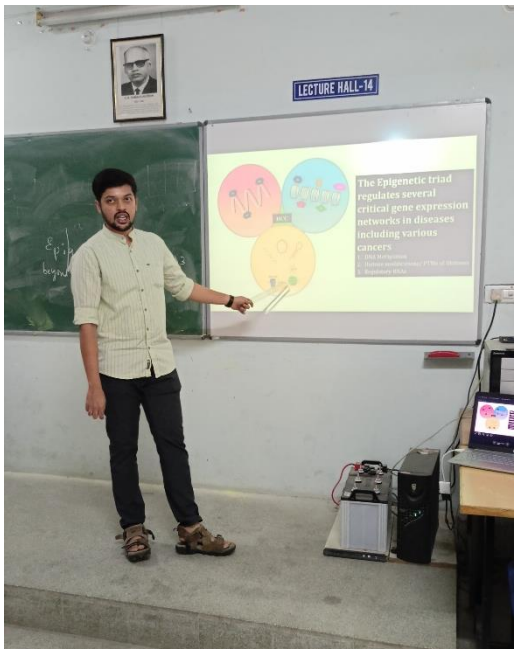
Given his interest in DNA methylation, he next artificially methylated the cloned vector using methyltransferase. Comparing the methylated and unmethylated vectors, he observed repression of luciferase activity in the methylated vector, confirming that methylation silences promoter activity. To further confirm this, he performed bisulphate conversion and lollipop diagram analysis, revealing hypermethylation in the CpG island region of the MEG3 promoter. This hypermethylation was associated with downregulation of both MEG3 and the miRNA cluster in HCC.

To explore the reversibility of methylation, he used the cytidine analogue drug decitabine for demethylation treatment. This resulted in the overexpression of MEG3 protein, demonstrating that methylation is a reversible process and that demethylation can restore gene function.

Conclusion

In conclusion, his studies identified the specific promoter region for the microRNA cluster and MEG3. The artificial methylation experiments confirmed that DNA methylation represses gene activity, while demethylation restored MEG3 expression, providing insight into the potential for epigenetic therapies in HCC. These findings underscore the critical role of DNA methylation in regulating the expression of tumor suppressor genes like MEG3 and their associated microRNAs in HCC progression.

Prof. N.S. Devaki welcomed the speaker and the audience to the program



Mr. Shreyas. H.K explaining about his work

Interaction session with Mr. Shreyas Hulusemane Karunakara

Report prepared by Rakshitha M, 5th year Integrated M.Sc. in Molecular Biology, Yuvaraja's College, Mysuru.

Date: 01/01/25

Alumni Interaction Session #4

[by Shreyas Hulusemane Karunakara]

Attendance Sheet

Sl.no.	Student Name	Department and semester	Signature
1.	Digna Mary Francis	Int. MSc Molecular Biology 9th Semester	Digna Mary
2.	V.R. Sampreethi Aradhya	Int. Msc. In Molecular Biology 9th semester	
3.	Navya · S.	Int.. msc. Molecular Biology 9th Semester	
4.	Yuvashree. K	Int. Msc. molecular Biology 9th Semester	
5.	Vaishnavi. S	Integ msc molecular Biology 9th Semester	Vaishnavi S
6.	~~Vandana P.M.~~		
7.	Poorvi S.	Int. MSc. Molecular Biology (9th semester)	Poorvi
8	Preethi · K	Int · MSc Molecular Biology 9th Semester	
9	Srinandini KR	Int. MSc Molecular Biology 9th Sem	
10	Ananya M. S	Int MSc in Molecular Biology, 9th sem	
11	Yashwanth. M	" "	
12.	Darshan M.N.	Integ. MSc Mol Bio 7th Sem.	
13)	Revanth Gowda S.R	Int. MSc. Mol. Bio 9th Sem.	
14	Vibha. V	Int. MSc Molecular Biology 7th sem	

Dr. Sampreethi M.S 01/01/25
Assistant Professor & Course Coordinator
Department of Molecular Biology
Maharani's Science College (Autonomous)
Maharani's Science College for Women, Mysore

Sl. no.	Student Name	Department and Semester	Signature
15	ANRUTH KUMAR. R K	Integrated M.Sc., Molecular biology	
16.	Supriya S Sundar	Integrated M.Sc Molecular Biology 7th sem	
17.	Srilakshmi. N	Integrated MSc. Molecular Biology 7th sem	
18	Bhoomika. B J	Int - Msc. Mol. Bio / 7th sem	
19	Aravaneth. K	Integrated MSc Molecular Biology 7th Semester	
20	Rakshitha. M	Int. Msc Molecular Biology 7th Sem	Rakshitha. M.
21.	Darshini. M.B	Int MSc Mol Bio / 7th Semester	
22.	Shyam Bharadwaj. D.M	Molecular biology / 7th Sem.	
23	Abhay. Mahesh. Baadkar	7th Semester, Molecular Biology	
24.	Vedashree. N. H	7th Sem / molecular Biology.	Vedashree. N.H.
25.	Uttam G	9th Semester, Molecular Biology	G. Uttam
26	Sanjana Shajan	Faculty - Molecular Biology integrated Msc.	
27	Dhanya. P	9th semester - molecular biology	Dhanya.
28.	Shreyas. H.K.	Invited Interaction, JSSMC	Shreyas H.K.
29	DEVAKI N. S	Faculty. Molecular Biology	Devaki. N. S 1/11/2025

HOD & Course Coordinator
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