CHAPTER 31

MEDICAL SCIENCES
BIOMEDICAL RESEARCH

Doctoral Theses

01. KOHLI (Surabhi)
Investigating the Genomic Correlates of Male Specific Chromatin Organization and Other Unique Phenomena in the Mealybug, Maconellicoccus Hirsutus.
Supervisor : Prof. Vani Brahmachari
Th 25334

Abstract
(Verified)

Mealybugs are invasive pests with a broad host range and widespread global distribution. The diploid genome consisting five chromosomal pairs (2n=10), lacks distinct sex chromosomes. The sex determination is associated with selective inactivation and heterochromatization of paternal genome in males as a result of imprinting. Thus making, it an interesting model to study imprinting mechanisms. Mealybugs exhibit many other distinctive biological characteristics including extreme sexual dimorphism, high radioresistance, meiotic drive and nested endosymbiosis. We explored different genes contributing this unique biology of mealybug, Maconellicoccus hirsutus by utilizing the genome sequence and annotation generated in lab. Comparative genome analysis with other insects revealed expansion of pesticide, radiation and desiccation resistance genes conferring them better adaptation in extreme environments. Circadian pathway and some epigenetic modifier genes were absent. DNA methylation machinery in mealybugs is complete except for de-novo DNA methyltransferase, DNMT3. The presence of key DNA repair genes with endosymbiotic contribution, complete antioxidant machinery and a robust telomere maintenance machinery suggests the basis of radiation resistance of the mealybugs. We performed transcriptome sequencing and differential gene expression analysis in M. hirsutus males and females. The genes showing enriched expression in males and females functionally correlated with their dimorphic behaviour and morphology as well as sex specific physiology. Molecular studies in mealybugs revealed presence of Nuclease Resistant Chromatin (NRC), an unusual chromatin organization resistant to micrococcal nuclease, specifically associated with males. We performed DNA sequencing and proteomics analysis to examine this ribonucleoprotein fraction for its role in heterochromatization in mealybugs. We identified specific DNA sequence motifs and heterochromatin related proteins associated with NRC, suggesting its potential role in male specific facultative heterochromatin formation in mealybugs.

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associated with male specific chromatin organization in mealybugs. References. Appendices and List of publication

02. MUKESH KUMAR

Deciphering the Functional Dynamics of HDAC9 and Its Implication in Neuronal in Neuronal Pathology Following Cerebral Ischemia-Reperfusion Injury.

Supervisor : Dr. Anju Katyal

Th 25325

Abstract
(Not Verified)

Cerebral ischemia-reperfusion injury (IR) primes a cascade of events ranging from microvascular dysfunction to inflammatory sequalae or extensive neurological damage. Treatment of IR currently relies on supportive maneuverers since no specific target oriented therapy is successful. One of the bottlenecks in devising stroke therapeutics is lack of appropriate in-vivo models exactly mimicking the real life stroke situations and also the absence of a well characterized in-vitro model. Therefore, a strategy for in-vitro differentiation of Neuro-2a was elaborated. The culture conditions of reduced serum conc. and all-trans-retinoic acid treatment were effective in inducing differentiation of Neuro-2a. Further, it was found to be useful for in-vitro simulation of IR. A consistent loss of cholinergic indices such as α7-nAChR and ChAT were observed with the progression of ischemic pathology in C57BL/6J mice. In addition, Up-regulation of HDAC9 splice variants i.e HDAC9.1 and HDAC9.2 were found to be associated with decreased expression of ChAT. Inhibition of HDAC9.1 expression was found to be effective in preservation of neuronal architecture in CA1 region of the hippocampus as compared to inhibition of HDAC9.2. The preservation of the neuronal architecture was related to significant decrease in cellular apoptosis of the hippocampal neuronal populations. The molecular level findings were well correlated with the behavioral paradigm analysis. Upon dissecting the associated molecular signalling, a regulation of CHAT in a HDAC9 dependent manner was observed. Also, HIF-1α stabilisation was identifed as important event associated with HDAC9 overexpression. Prevention of p-mTOR, NF-κβ, i-NOS activation was found to be associated with prosurvival response seen after HDAC9.1 inhibition. Overall, in the present study, the function of HDAC9 in IR pathogenesis was elucidated and a distinct importance of HDAC9 splice variants in instigating the molecular networks associated with pathological sequalae was established.

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03. UBOVEJA (Apoorva)
Identification of P73 Mediated Regulation of Novel Molecular Targets That Aid in the Suppression of Cancer Cell Migration Invasion and Metastasis.
Supervisor : Prof. Daman Saluja
Th 25337

Abstract
(Not Verified)

p73 belongs to the p53 tumor suppressor family and can transactivate p53-responsive genes. As p73 is rarely mutated in cancers in contrast to p53, it holds a key to therapeutic benefits. In this study, we have investigated the underlying mechanisms that aid in the anti-metastatic functions of p73. We have identified Neuron Navigator-3, a microtubule-binding protein, as a novel transcriptional target of p73, which gets up-regulated by DNA damage in a p73-dependent manner. Knockdown of NAV3 enhanced the migration and invasion rates of colorectal cancer cells in a p73 dependent manner. Immuno-histochemistry analysis demonstrated that both NAV3 and p73 are down-regulated in metastatic colon cancer tissues as compared to non-metastatic tissues. This study bestows conclusive evidence that Neuron Navigator-3 portrays an important role in p73-mediated metastasis inhibition. We have also employed transcriptome sequencing to identify various long non-coding RNA's that are up-regulated or down-regulated in the presence of p73. LncRNA FER1L4 was discovered as a novel p73 transcriptional target and p73 binding onto FER1L4 promoter was confirmed through bioinformatics analysis, luciferase reporter assay, ChIP and site-directed mutagenesis assays. Knockdown of FER1L4 and p73 enhanced the invasion and migration rate of colorectal cancer cells. FER1L4 plays a pivotal role in p73 mediated regulation of cell cycle arrest and apoptosis. FER1L4 also sponges the oncogenic action of miR-1273g-3p. RNA-ISH experiments revealed down-regulation of FER1L4 mRNA and p73 mRNA in metastatic colorectal cancer tissue samples as compared to non-metastatic samples. Taken together, we provide conclusive proof that p73 exerts its anti-metastatic function by inducing FER1L4 expression in response to genotoxic stress.

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04. YADAV (Sonal)
Differential Expression of Toll like Receptor and NOD like Receptor in Response to Symptomatic and Asymptomatic Trichomonas Vaginalis Infections.
Supervisor : Dr. Manisha Yadav
Th 25336

Abstract
(Verified)

Trichomonas vaginalis is a parasitic protozoan that causes a sexually transmitted disease called trichomoniasis. Innate immune system is the first line of defense against pathogens, acts via receptors like Toll-like receptors (TLRs) and NOD-like receptors (NLRs). The involvement of NLRP3, TLR2, TLR4, and TLR9 in trichomoniasis has been discussed in recent studies. There is a lack of research in the role of NLRs (NLRP3, NLRC4), non-NLR (AIM-2), and TLRs (TLR1/2, TLR4, TLR5, and TLR9) in response to symptomatic and asymptomatic T. vaginalis infections. Therefore, in the present study we have investigated the expression of the NLRs (NLRP3, NLRC4, AIM-2), and TLRs (TLR1/2, TLR4, TLR5, and TLR9)

by quantitative real-time PCR and immunohistochemistry in the vagina and cervix tissues of BALB/c mice infected with symptomatic and asymptomatic T. vaginalis isolate. We found a higher expression of Tlr1, Tlr4, Tlr9, Nlrc4, Nlrp3, and Aim-2 in the cervical tissues at later time points in asymptomatic groups. Elevated Nlrp3 expression was observed at early time points in the symptomatic group. In comparison, increased Nlrp3 and Nlrc4 levels were seen in asymptomatic groups, i.e., delayed expression was observed in the cervical tissues. In the vaginal tissue, elevated Tlr1/Tlr2 was observed at early time points in the symptomatic group. However, Tlr4 was measured in the asymptomatic group at early time points. We conclude from this study that differential expression of TLRs and NLRs was observed in the vaginal and cervical tissues of BALB/c infected with symptomatic and asymptomatic isolates of T. vaginalis. Our finding suggests that TLRs and NLRs play an important role in recognizing ligands of T. vaginalis infection.

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