Chapter 8

SUMMARY AND CONCLUSIONS

This study uncovered the critical role two diverse groups of epigenetic regulators such as Jumonji domain containing histone lysine demethylases (in particular KDM4/JMJD2 family members) and NAD-dependent histone lysine deacetylases (in particular SIRT1 and SIRT2) play in mediating the effects of chronic social defeat stress on adult hippocampal neurogenesis leading to depression, anxiety and related mood disorders.

This is first such study where the role of epigenetic regulators such as Sirtuins has been uncovered even during the onset of depression and anxiety, i.e. at day 5 of the chronic defeat paradigm, in addition to usual 10 days of social defeat stress paradigm, which induces mood disorders like phenotype.

The mood disorders in mouse model appear to be due to the adverse effect of chronic stress on the structural plasticity of dentate gyrus neurons, as evident by the analysis of Golgi-Cox study.

10 days of defeat stress led to significant reduction in proliferating neural stem /and or cell (NSCs/NPCs) populations in dentate gyrus, as measured by BrdU and Nestin counts in C57bl/6 mice and Nestin-GFP transgenic reporter mice, respectively.Chronic stress also led to significant decrease in the neuronal differentiation, as evident bytheattenuation of doublecortin (DCX) +vecells in DG,the early marker of neuronal differentiation.

The qPCR, WB, DMOG studies using both *in vivo* and *ex vivo* system helped us to conclude the crucial role Jmjd2C and Jmjd2Dplayed in mediating the effects of chronic stress on adult hippocampal neurogenesis leading to depression, anxiety and related mood disorders.

This insight also gives us therapeutic strategy for the treatment of mood disorders by developing small molecules that can activate these Jmjds or inhibit its epigenetic target H3K9me2.

The other class of epigenetic regulators, NAD+dependent ‘Sirtuins’, in particular few of the nuclear sirtuins, also appear to be implicated in our repeated stress-induced depression and related mood disorders model. Out of four nuclear Sirtuins, Sirt1 and Sirt2 was found to be

dysregulatedin the neurogenic DG both during the onset of depression and also at full-blown depression.

The *ex vivo* studies using primary neurosphere cultures led us to uncover the crucial role Sirt1 and Sirt2 play in controlling neurogenesis, i.e. proliferation and differentiation. The level of Sirt1 and Sirt2 were opposite to each other; Sirt1 mRNA and protein level was higher in proliferating NPCs compared to its level in differentiation stage, whereas Sirt2 mRNA and protein level was higher in differentiating phase compared to its level during proliferation.

Sirt1 acts epigenetically (by deacetylating histone H4K16) and the analysis of chromatin immunoprecipitation (ChIP) and qPCR data from the proliferating and differentiating DG cultures suggest Sirt1 regulation of a number of gene promoters such as Stat1, Egr3, Dnmt3a and E2f1, which play crucial role in controlling proliferation and differentiation, or neurogenesis.

Thus, we report another novel finding i.e. gene targets through which Sirt1 plays its epigenetic regulatory function in chronic stress affected neurogenesis and the etiopathology of affective disorders.

The viral-mediated gene targeting experiments using *ex vivo* as well as *in vivo* system suggest that Sirt2 is also an important player in the etiopathology of mood disorders (although it doesn't work epigenetically). Sirt2 might be acting this by deacetylating the non-histone proteins, in particular the tubulin (by regulating its acetylation and hence polymerization), crucial for cytoskeletal reorganization that is indispensable for the differentiation of NSCs/NPCs.

Another novel finding we report here is the implication of Sirt6 in the neural stem or progenitor cells' proliferation and differentiation, as shown by our *ex vivo* experiments.

The novel outcome of the study on nuclear sirtuins in our diverse models might have therapeutic implication.

Lastly my research effort, together with that of my colleagues and collaborating laboratories, resulted in zeroing in onto two novel compounds with remarkable neurotrophic and/or neurogenic property and one of these i.e. compound#1 showed great potential for the therapeutic development to treat acute ischemic stroke (AIS).

This was an endeavor to develop epigenetic mechanism based therapeutics development where the idea was to identify novel molecules, out of the available compound libraries at our institute, and evaluate whether these chemical entities modulate nuclear sirtuins and/or histone acetylation. So, using *in vitro*, *ex vivo*and *in vivo* modelswe discovered two interesting compounds that not only modulated the levels of Sirtuin 1 and 2, in addition to its role in TrkB-MEK-ERK-CREB signaling mechanism, but also showed neuroprotection in AIS model. Further studies will be required to analyze the efficacy of chronic treatment with the more neurogenic compound#2 in animal models of diverse brain and behavior disorders where the neurogenesis is severely affected, including neurodegenerative disorders and psychiatric disorders such as depression and PTSD.