Epigenetic Regulation by the histone "Reader/Effector" proteins and its implication in human diseases:

The focus of Dr. Das' laboratory has been to understand the underlying epigenetic regulations that play a pivotal role in fine-tuning gene expression programs in human diseases. Central to her research is the study of chromatin reader/effectors, a class of proteins that recognize specific epigenetic landscapes and differentially regulate gene expression programs under physiological and pathophysiological conditions.

Epigenetic regulation in cancer

A) Triple Negative Breast Cancer

1. Adhikary S., Chakravarti D., Terranova C., Sengupta I., Maitituoheti M., Dasgupta A., Srivastava D.K., Ma J., Raman A.T., Tarco E., Sahin A. A., Bassett R., Yang F., Tapia C., Roy S.*, Rai K.*, **Das C*. *2019.*** Atypical Plant Homeodomain of UBR7 Functions as an H2BK120Ub Ligase and Breast Tumor Suppressor. ***Nat Commun.*** 10(1):1398.

Seminal Discovery: We have identified that the PHD finger of UBR7 have a unique function of enzymatic catalysis of histone H2BK120Ub. Further we establish the mechanism by which UBR7 can suppress *triple negative breast cancer proliferation and metastasis*. While the basic mechanism *in vitro* and *ex vivo* was established in our laboratory, global gene targets identification by integrated ChIP-Seq and RNA-Seq analysis, *in vivo* validation in nude mice models and cohort of TNBC sample analysis (where functional score has been assigned by doctors and statisticians) were done in collaboration. *Collectively, UBR7 has been established as a novel histone H2B monoubiquitin ligase that suppresses tumorigenesis and metastasis in TNBC.*

2. Adhikari S., Singh V., Nandi S., Ghoshal M., Sundar Raj N., Khanna J., Bhattacharya A., Kabiraj A., Mondal A., Vasudevan M., Senapati D., Roy H., Sengupta K., Notani D., **Das C.*** ***2024***. UBR7 in concert with EZH2 inhibits the TGF-β signalling leading to extracellular matrix remodelling. ***Cell Reports*** 43(7):114394.

Seminal Discovery: The intricate interplay between resident cells and the extracellular matrix (ECM) profoundly influences cancer progression. We have identified a crucial mechanism where epigenetic factors, UBR7 and EZH2 regulate TGF-β/Smad signaling, affecting the expression of ECM genes. UBR7 loss dramatically reduces H3K27me3 mark activating ECM genes. We have observed that UBR7 plays *a crucial role in matrix deposition in adherent cancer cells and spheroids, altering collagen content and lysyl oxidase activity, directly affecting matrix stiffness and invasiveness*. These results could be confirmed in mouse models of breast tumor as well as TNBC patient samples. Thus reduced *UBR7 levels are accompanied by increased ECM component expression and activity, leading to fibrosis-mediated matrix stiffness. Collectively, UBR7 is a master regulator of matrix stiffening, influencing the metastatic potential of TNBC.*

3. Mukherjee S., Adhikary S., Gadad S.S., Mondal P., Sen S., Choudhari R., Singh V., Adhikari S., Mandal P., Chaudhuri S., Sengupta A., Lakshmanaswamy R., Chakrabarti P., Roy S., **Das C*. *2020.*** Suppression of poised oncogenes by ZMYND8 promotes chemo-sensitization. ***Cell Death Dis.*** 11(12):1073.

Seminal Discovery: We have established a new mechanism by which we show that the tumor promoting genes are maintained in a poised or bivalent epigenetic state in triple

negative breast cancer. Further. We show that the chromatin reader protein ZMYND8 can chemosensitize cells to repress the tumor promoting genes when sub-lethal dose of chemotherapeutic drugs is provided to the TNBC cells. We validate this mechanism in vivo mice tumor model in collaboration. *Collectively, our research findings indicate that poised chromatin is instrumental for acquisition of chemo-resistance by cancer cells and propose ZMYND8 as a suitable epigenetic tool that can re-sensitize the chemo-refractory breast carcinoma.*

B) Neuroblastoma

4. Adhikary S., Singh V., Choudhari R., Yang B., Adhikari S., Ramos E.I., Chaudhuri S., Roy S., Gadad S.S.*, **Das C.*** ***2022.*** ZMYND8 suppresses MAPT213 LncRNA transcription to promote neuronal differentiation. ***Cell Death Dis.*** 13(9):766.

Seminal Discovery: We have established that ZMYND8 promotes neuronal differentiation by positively regulating canonical MAPT protein-coding gene isoform, a key player in the axonal development of neurons. Interestingly, ZMYND8 modulates *gene-isoform switching* by epigenetically silencing key regulatory regions within the MAPT gene, thereby suppressing the expression of *non-protein-coding isoforms such as MAPT213*. Genetic deletion of ZMYND8 led to an increase in the MAPT213 that potentially suppressed the parental MAPT protein-coding transcript expression related to neuronal differentiation programs. *Collectively, our results elucidate a novel mechanism of ZMYND8-dependent transcription regulation of different neuronal lineage committing genes, including MAPT, to promote neural differentiation.*

5. Adhikary S., Sanyal S., Basu M., Sengupta I., Sen S., Srivastava D.K., Roy S.*, **Das C.*** ***2016.*** Selective Recognition of H3.1K36 dimethylation / H4K16 acetylation facilitates the regulation of ATRA-responsive genes by putative chromatin reader ZMYND8. ***J Biol Chem.***, 291:2664-2681.

Seminal Discovery: In this study we established that through its specific key residues present in its conserved chromatin-binding modules, ZMYND8 interacts with the selective epigenetic marks H3.1K36Me2/H4K16Ac. Furthermore, ZMYND8 shows a clear preference for canonical histone H3.1 over variant H3.3. nterestingly, ZMYND8 was found to be recruited to several developmental genes, including ATRA-responsive genes through its modified histone-binding ability. *Overall, our study identifies that ZMYND8 has NuRD-independent functions in regulating gene expression through its modified histone-binding ability.*

Epigenetic regulation in HBV infection and HBV-induced HCC

6. Singh V., Mondal A., Adhikary S., Mondal P., Shirgaonkar N., DasGupta R., Roy S., **Das C.*** ***2024***. UBR7 E3 Ligase suppresses IFN-β mediated immune signaling by targeting Sp110 in HBV-induced HCC. ***ACS Infectious Diseases*** (In Press). (*Accepted for Cover Page Publication*)

Seminal Discovery: Viruses often hijack the cellular factors to promote their proliferation in host cells. Hepatitis B virus (HBV), belonging to Hepadnaviridae family, remains undetected in early infection as it does not induce the innate immune response and can lead to cirrhosis and hepatocellular carcinoma (HCC). Atypical PHD finger of ubiquitin protein ligase E3 component n-recognin 7 (UBR7), plays a crucial role in histone H2BK120 monoubiquitination. *We have identified a new function of UBR7 in Hepatitis-B virus (HBV) pathogenesis in the context of factor ubiquitination.* Notably, Speckled 110 (Sp110) has been previously shown to be a resident of PML-

NB, where it remains SUMOylated, and during HBV infection, it undergoes deSUMOylation and exits the PML body. We observe that UBR7 ubiquitinates Sp110. Remarkably, Sp110 ubiquitination *downregulates genes in the type I interferon response pathway*. We observed that silencing UBR7 induces IRF7 phosphorylation, thereby augmenting IFN-β and the downstream interferon-stimulated genes (ISGs). *Our study establishes a new function of UBR7 in non-histone protein ubiquitination, promoting viral persistence.* Thus, *we elucidate a mechanism by which HBV can evade host immune response by hijacking these epigenetic regulators* which has immense potential in the antiviral therapy.

7. Sengupta I., Mondal P., Sengupta A., Mondal A., Singh V., Adhikari S., Dhang S., Roy S., **Das C.*** ***2022.*** Epigenetic regulation of Fructose-1,6-Bisphosphatase 1 by host transcription factor Speckled 110 kDa during Hepatitis B Virus infection. ***FEBS J.*** 289(21):6694-6713.

Seminal Discovery: Our study accentuates the interplay between epigenomic alteration and metabolic reprogramming due to Hepatitis B virus infection. Gluconeogenesis, which is mainly controlled by rate-limiting enzymes PCK1, FBP1, and G6PC, is usually downregulated in cancers. An induction of gluconeogenesis by HBV intrigued us to look into the mechanism of its regulation. Here we report a novel mechanism of epigenetic regulation of FBP1, by HBV via exploiting the chromatin reader function of the host factor SP110. We identified histone H3K18Ac as an epigenetic mark specifically recognized by SP110-Bromodomain. We unraveled that this interaction leads to the recruitment of SP110 on the H3K18Ac enriched FBP1 promoter. In presence of HBV, deacetylase SIRT2 is recruited onto FBP1 promoter in an SP110 dependent manner and removes the epigenetic mark from the site, thereby altering its expression. Importantly, this Sp110-mediated metabolic regulation during infection has a direct consequence in viral-borne hepatocellular carcinoma (HCC). *Collectively, we propose that the epigenetic reader protein Sp110 has immense potential to be a therapeutic target to challenge HBV-induced HCCs by modulating the metabolic programs.*

8. Sengupta I., Das D., Singh S.P., Chakravarty R., **Das C.*** ***2017.*** Host transcription factor Speckled 110 kDa (Sp110), a nuclear body protein, is hijacked by Hepatitis B virus protein X for viral persistence. ***J Biol Chem.,*** 292(50):20379-20393.

Seminal Discovery: We have established in this study that the PML-NB protein Speckled 110 kDa (Sp110) is SUMO1-modified and undergoes a deSUMOylation-driven release from the PML-NB in the presence of HBV. Intriguingly, Sp110 knockdown significantly reduced viral DNA load in the culture supernatant by activation of the type I interferon-response pathway. Furthermore, we found that Sp110 differentially regulates several direct target genes of hepatitis B virus protein X (HBx), a viral co-factor. Subsequently, we identified Sp110 as a novel interactor of HBx and found this association to be essential for the exit of Sp110 from the PML-NB during HBV infection and HBx recruitment on the promoter of these genes. HBx, in turn, modulates the recruitment of its associated transcription cofactors p300/HDAC1 to these co-regulated genes, thereby altering the host gene expression program in favor of viral persistence. *Our results collectively delineate a mechanism by which HBV can evade host immune response by hijacking the PML-NB protein Sp110, and therefore, we propose it to be a novel target for antiviral therapy.*

Epigenetic regulation in maintenance of metabolic homeostasis

9. Mondal P., Gadad S.S., Adhikari S., Ramos E.I., Sen S., Prasad P., **Das C*. *2021.*** TCF19 and p53 Regulate Transcription of TIGAR and SCO2 in HCC for Mitochondrial Energy Metabolism and Stress Adaptation. ***FASEB J.*** 35(9): e21814.

Seminal Discovery: In this study we have elucidated that TCF19 interacts with a non-histone, well-known tumor suppressor protein 53 (Tp53) and co-regulates a wide array of metabolic genes. Among these, the p53-responsive carbohydrate metabolic genes, TIGAR and SCO2, which are the key regulators of glycolysis and oxidative phosphorylation, are under direct regulation of TCF19. Remarkably, TCF19 can form different transcription activation/repression complexes which show substantial overlap with that of p53, depending on glucose-mediated variant stress situations as obtained from IP/MS studies. Interestingly, we observed that TCF19/p53 complexes either have CBP or HDAC1 to epigenetically program the ex- pression of TIGAR and SCO2 genes depending on short-term high glucose or prolonged high glucose conditions. TCF19 or p53 knockdown significantly altered the cellular lactate production and led to increased ECAR. Similarly, OCR and cellular ATP production were reduced and mitochondrial membrane potential was compromised upon depletion of TCF19 or p53. *Together, the study proposes that TCF19/p53 complexes can regulate metabolic gene expression programs responsible for mitochondrial energy homeostasis and stress adaptation.*

10. Sen S., Sanyal S., Srivastava D.K., Dasgupta D., Roy S., **Das C.* *2017.*** Transcription factor 19 regulates gluconeogenesis in concert with the nucleosome-remodelling-deacetylase complex via histone 3 lysine 4 trimethylation recognition. ***J Biol Chem.,*** 292(50):20362-20378. (*Accepted for Cover Page Publication*).

Seminal Discovery: In this study we established that TCF19 selectively interacts with histone 3 lysine 4 trimethylation through its plant homeodomain finger. Knocking down TCF19 under high-glucose conditions affected many metabolic processes, including gluconeogenesis. We found that TCF19 overexpression represses de novo glucose production in HepG2 cells. The transcriptional repression of key genes, induced by TCF19, coincided with NuRD complex recruitment to the promoters of these genes. *In summary, our results show that TCF19 interacts with an active transcription mark and recruits a co-repressor complex to regulate gluconeogenic gene expression in HepG2 cells. Our study offers critical insights into the molecular mechanisms of transcriptional regulation of gluconeogenesis and into the roles of chromatin readers in metabolic homeostasis.*