Statement of research achievements, if any, on which any award has already been received by the applicant. Please also upload brief citation(s) on the research work(s) for which the applicant has already received the award(s) (not to exceed 2000 words)

CDRI Award 2021 for Excellence in Drug Research in Life Science category, from Council of Scientific & Industrial Research (CSIR)
Award Citation- Decoding the Epigenetic Landscape by the 'Histone Readers': Implications in Human Diseases: Epigenetic modifications are recognized by a ubiquitous class of proteins called "readers/effectors" which has become an important paradigm in chromatin biology. We have observed that chromatin readers are intricately involved in reprogramming the epigenetic landscape of chromatin thereby having important role in human diseases including cancer, infectious diseases and metabolic disorders. Furthermore, this class of proteins have immense potential that can be exploited in the therapeutic regimen.

S. Ramachandran - National Bioscience Award For Career Development-2019, from Department of Biotechnology (DBT)
Award Citation- Investigating the functional interplay between key transcription factor TCF7l2 and epigenetic regulator TCF19 to modulate metabolic gene expression programs during Endoplasmic Reticulum stress: The goal of the present project is to investigate the co-regulated processes between an important epigenetic regulator TCF19 and a gluconeogenic regulator and classic transcription factor TCF7l2 and how the interplay of these factors effects hepatic Insulin Resistance (IR) through transcriptional regulation during ER stress.

SwarnaJayanti Fellowship (2017-2018) from Department of Science and Technology (DST)
Award Citation- Reprogramming of Host Epigenomic landscape during viral infection: The overall goal of the project is targeting the epigenetic landscape of the host metabolic genes to challenge viral proliferation.

Ramalingaswami Fellowship (2011-2012) from Department of Biotechnology (DBT)
Award Citation- Prolyl isomerization as a novel mode to regulate chromatin function: The goal of this project was to understand the role of critical residues of CBP and Pc2 involved in crucial chromatin-mediated functions and consequent human diseases (Rubinstein Tybi Syndrome and Cancer).

Details of the research work duly signed by the applicant, for which the Sun Pharma Science Foundation Research Award is claimed, including references and illustrations (not to exceed 6000 words).

Introduction:

Breast cancer has been the leading cause of cancer mortality among females of all ages worldwide. The heterogeneity of the disease, globally topping one-million a year, poses immense challenge in deciphering new therapeutic strategies. Hormone-receptor negative subtype has the worst prognosis due to lack of proper molecular classifications. Apart from accumulation of genetic defects, epigenetic abnormalities play a significant role in initiation, progression and metastases of breast cancer. Epithelial to mesenchymal transition (EMT), which preludes the onset of metastasis is also driven by epigenetic alterations. With the initiation of breast cancer there is loss of H3K4Me2/H3K9Ac, important for tumor maintenance. Importantly, breast cancer with worst prognosis shows a lower level of H3K18Ac/H4K12Ac/H3K4Me2/H4K20Me3/H4R3Me2 marks. In this context, histone monoubiquitination is of prime importance as it is instrumental in identifying new therapeutic strategies for a better disease-free survival. Notably, several histone E3 ubiquitin ligases have been implicated in breast cancer either promoting or suppressing the disease. It has been recently established by our group that the chromatin readers (ZMYND8/ CBX4/ TCF19/ Sp110) recognize and functionally interpret the histone PTMs employing distinct mechanism impacting the transcription-programs and has seminal role in regulating tumorigenicity.

Outstanding Discoveries:

A family of mammalian E3 Ubiquitin ligases UBR1 -UBR7, characterized by a 70-residue zinc finger type UBR-box domain, is essential for recognition of the N-degrons. Interestingly, UBR7 has evolved with a Plant Homeodomain (PHD) finger, unique among UBR family members. Although PHD finger is well characterized for methylated or unmodified histone H3 binding ability, no previous reports of its E3 Ubiquitin ligase activity exist. We demonstrated that the PHD finger of Ubiquitin Protein Ligase E3 Component N-Recognin7 (UBR7) harbors E3 ubiquitin ligase activity toward monoubiquitination of histone H2B at lysine120 (H2BK120Ub). It was found through *in vitro* ubiquitination assay, that isolated PHD finger as well as full-length wild-type UBR7 could monoubiquitinate histone H2B at lysine120. UBR7 loss dramatically reduces H2BK120Ub levels both globally and genome wide on cell-cell adhesion linked genes like CDH4. Interestingly, it was found that UBR7 expression was negatively corelated with triple-negative breast cancer (in cell lines as well as patients' samples) and metastatic tumors (in mice). Similarly, UBR7 suppresses 2D proliferation, 3D colony formation and tumor formation *in vivo*. Again, suppression of invasiveness, migratory potential and lung metastasis *in vivo* confirmed the metastasis suppressive role of UBR7. It was identified that UBR7 loss promotes EMT and activates canonical Wnt/β-Catenin signaling pathway which marks the onset of metastasis. Finally, it was shown that CDH4/R-Cadherin is the direct target of UBR7 and complementing with CDH4 revert back phenotypes caused due to loss of UBR7. Collectively this study showed that UBR7 PHD finger monoubiquitinates H2BK120 at the gene body region of CDH4, thereby

maintaining the epithelial state of the cells and suppressing the metastasis of triple negative breast cancer. TNBC has the worst prognosis due to limitations in therapy. Since UBR7 is downregulated in TNBC, reinstating the protein can be implemented as a new therapeutic strategy, whereby UBR7 alters the epigenome via its catalytic function, thus suppressing tumor metastasis.

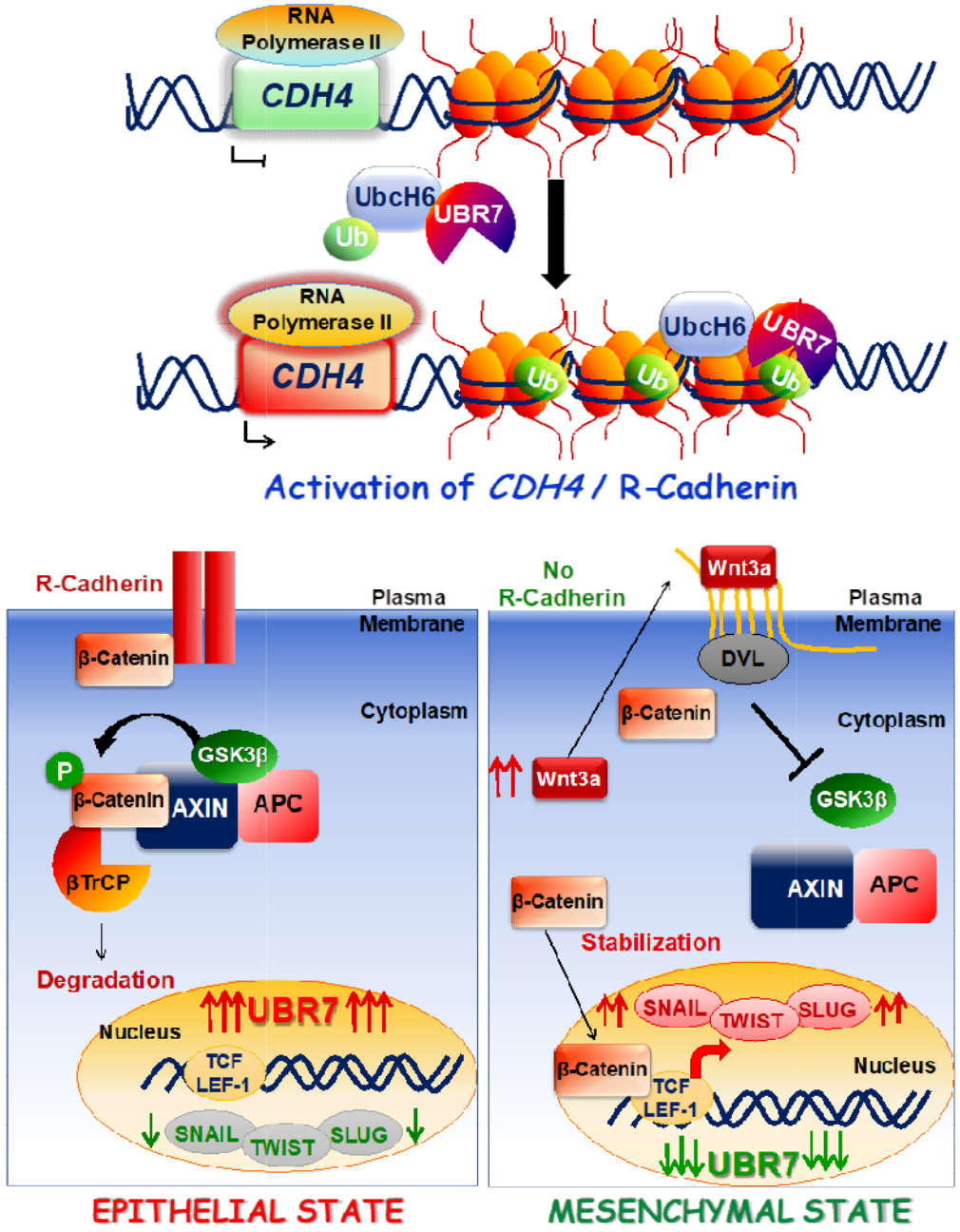

Figure Legend: Metastasis suppression through R-Cadhering gene expression regulation by E3 Ubiquitin ligase UBR7 by H2BK120 monoubiquitination.

In an attempt to further delineate the role of UBR7 in triple negative breast cancer metastasis, it was observed that this protein is uniquely involved in Extracellular Matrix (ECM) remodeling. The bidirectional interaction between resident cells and ECM reshapes the ECM architecture of the tumor microenvironment to favor the survival and distant metastasis in cancer. In solid cancer, tumor cells and their associated stromal cells can produce large quantity of ECM components (lysyl oxidase, fibronectin and, collagen etc.) rendering fibrosis mediated stiffness in matrix. It was deciphered that UBR7 transcriptionally regulates the TGF beta signaling through which it influences the expression of different ECM genes in both 2D adherent cells and tumor sphere models. It was elucidated that UBR7 in coordination with EZH2 represses the expression of TGF beta pathway genes and influences its downstream target ECM genes by stabilizing the facultative heterochromatin mark H3K27me3. Besides regulating the ECM component deposition by cancer cells, UBR7 impacts ECM both biochemically and biophysically which have been validated by measuring lox activity and total collagen content from breast cancer patient tissue and an inverse correlation can be drawn between UBR7 expression and matrix stiffness. Finally, the correlation between UBR7 and different ECM genes from breast cancer patient samples has been delineated. Therefore, the deregulation of ECM genes by UBR7 alters matrix stiffness and fibrotic phenotype impacts invasion and metastasis in TNBC. Overall, these observations indicate that UBR7 can be a potential therapeutic target to overcome the challenge of matrix hardening driven cell survival and metastasis in TNBC.

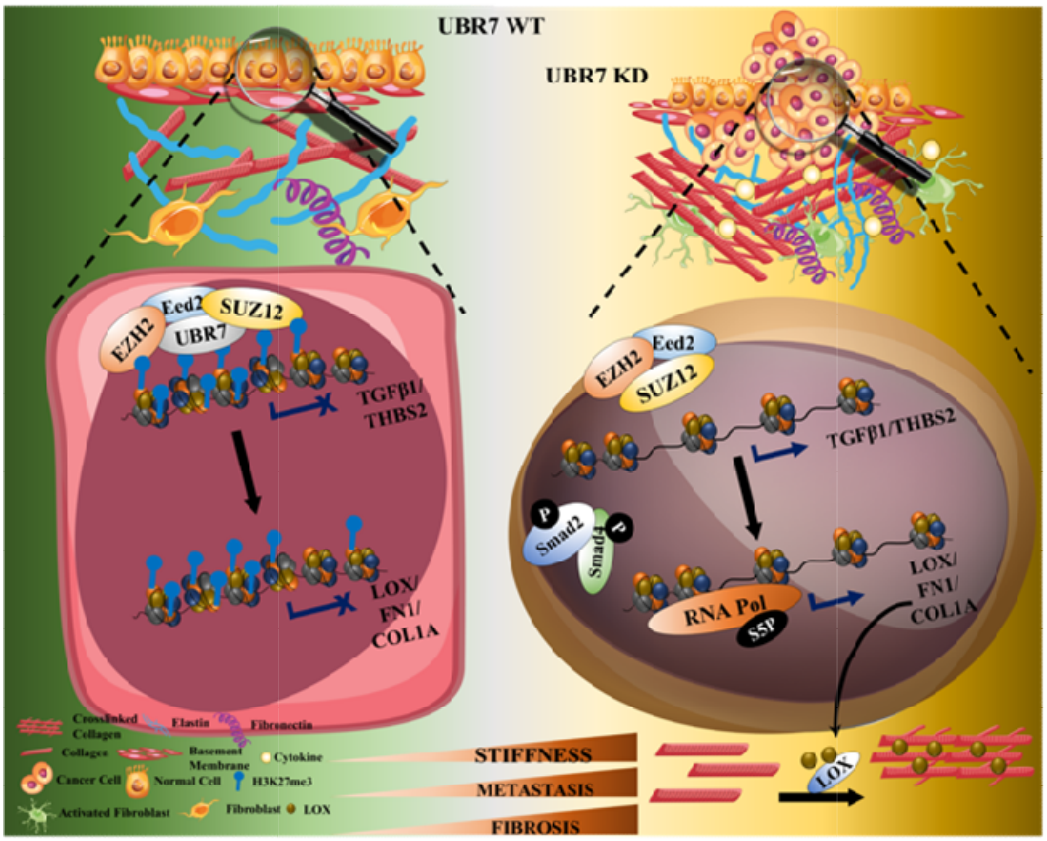

Figure Legend: Remodeling of Extracellular Matrix by UBR7 through selective epigenetic reprograming.

Manuscript Published:
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.

Manuscript Under Consideration:
Adhikari S., Bhattacharya A., Singh V., Khanna J., Ghoshal M., Mondal A., Nandi S., Kabiraj A., Nidharshan S.R., Adhikary S., Antariksh V., Vasudevan M., Senapati D., Roy H., Roy S., Sengupta K., Notani D., **Das C.*** 2023. Remodeling of extracellular matrix by chromatin regulator UBR7 in Triple-negative breast cancer: insights into stiffness and metastasis. (*Manuscript Under Consideration*)

Chandrima Das

14.8.2023
Signature of nominee with date