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.

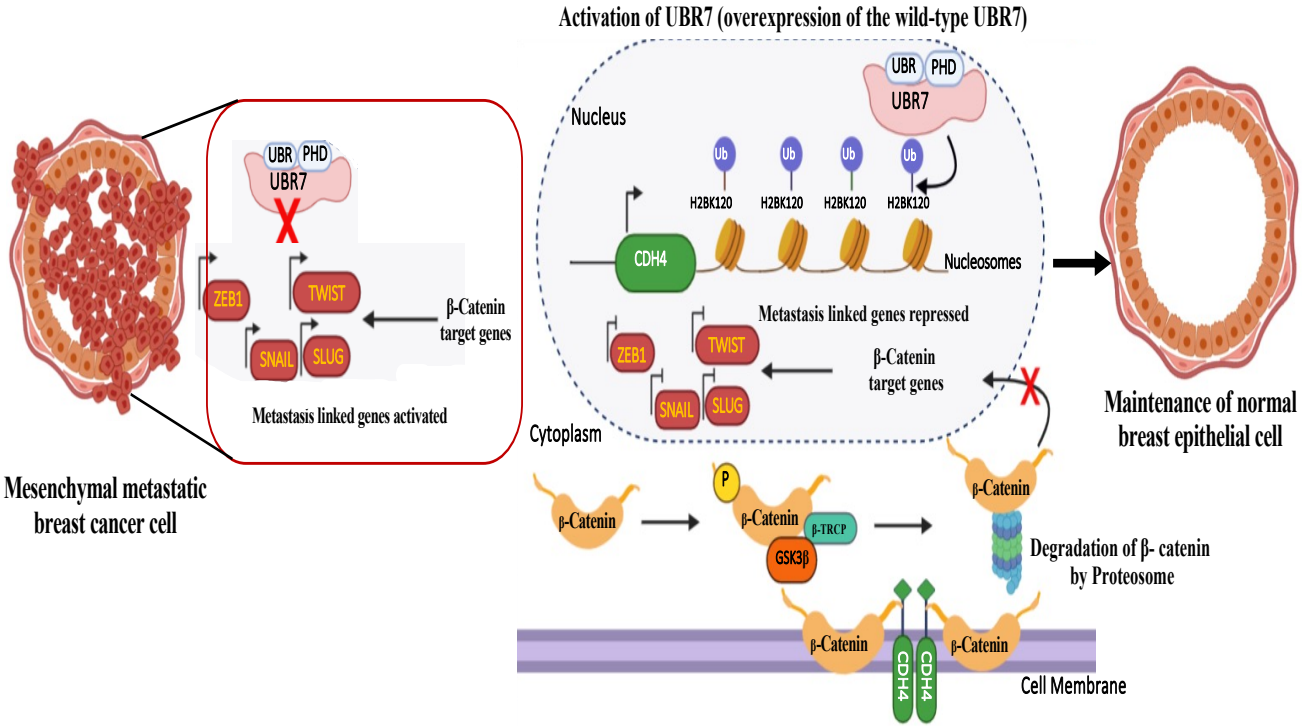

Figure 1: Metastasis suppression through R-Cadherin gene expression regulation by E3 Ubiquitin ligase UBR7 by H2BK120 monoubiquitination (*Nat Commun., 2019*).

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.

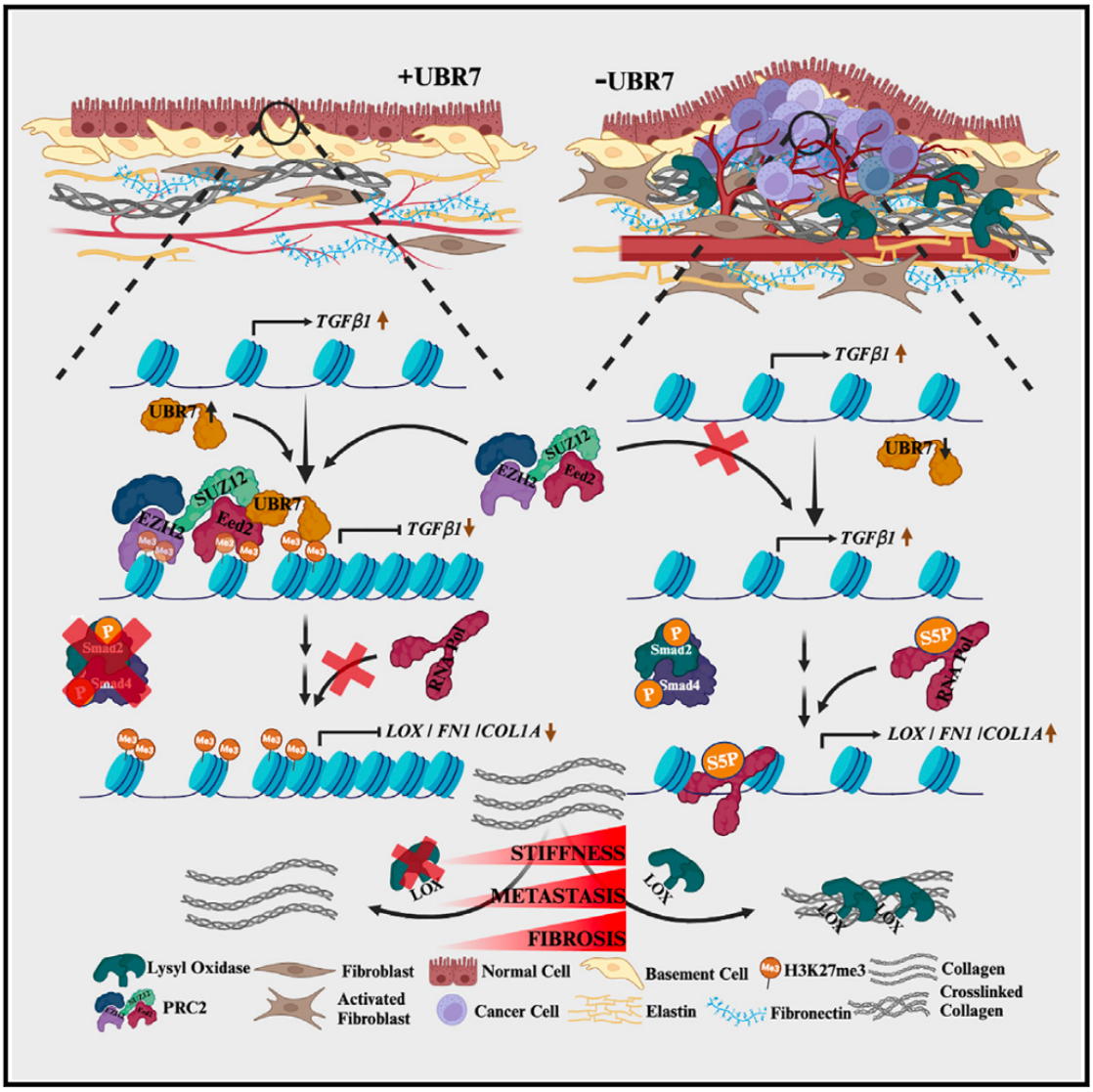

Figure 2: Remodeling of Extracellular Matrix by UBR7 through selective epigenetic reprograming (*Cell Reports, 2024)*.

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.

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.

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26.8.2024

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Signature of nominee with date