Role of PRMT5 in ULK1-mediated Autophagy in Breast cancer Therapy

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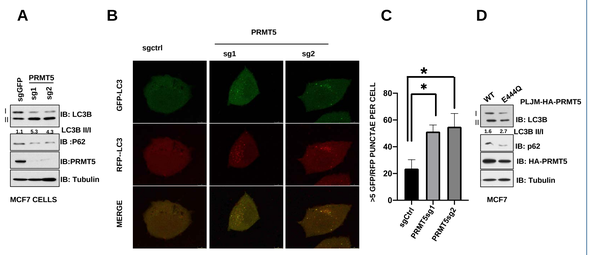
BACKGROUND

In mammals, a family of nine enzymes termed protein arginine methyltransferases (PRMT) catalyzes three types of arginine methylation including monomethyl arginine (MMA), symmetrical dimethyl arginine (sDMA) and asymmetrical dimethyl arginine (aDMA)[1,2]. PRMT5 is the dominant type II enzyme, which generates most of the sDMA on diverse nuclear and cytoplasmic substrates to control many critical cellular processes including transcription, DNA damage repair and signaling transduction[3]. Notably, PRMT5 is overexpressed in more than 50% of primary breast tumors and 70% of metastatic breast tumors, with strongest expression in triple negative type breast cancer (TNBC). Moreover, increased expression of PRMT5 is also associated with poor prognosis and survival in breast cancer patients[5,6]. Thus, PRMT5 has gained traction as an attractive target for breast cancer therapy. Currently, there are three PRMT5 inhibitors in clinical trials[7] including phase II trial on early-stage breast cancers (NCT04676516). Despite these significant advances in targeting PRMT5 for cancer therapy, the exact mechanism by which PRMT5 inhibition suppresses cancer progression is largely unknown. Moreover, preclinical study showed that compared to 2-day treatment for most inhibitors, more than 4-day treatment is required for PRMT5 inhibitor to achieve anti-proliferative effect in most cancer cells[8], indicating that a resistant mechanism may be induced at the early-stage treatment .Demystifying this mechanism could potentially reveal targetable avenues to improve PRMT5 inhibitors-mediated chemotherapy.

METHODS

CRISPR knockout and Stable cell line Generation
Lentiviral and retroviral packaging and subsequent infection were performed as described [9]. Selection and maintenance of cells were with hygromycin (250μg/ml), blasticidin(20μg/ml) and puromycin (1μg/ml).
Immunofluorescence
GFP-RFP-LC3B stable cells after PRMT5 knockout were grown on glass coverslips and fixed with 4% paraformaldehyde for 15 min at room temperature, washed three times with PBS, and then permeabilized with 0.05% Triton X-100 for 10 min at room temperature. Following three washes of 5 min in PBS, the coverslips Images taken photographed at random positions for each condition.
Immunoblot and Immunoprecipitation
Cells were lysed and lysates incubated with 50% slurry of agarose conjugated antibody for 3–5 h at 4 ° C. The immunoprecipitate were washed four times with NETN buffer (20 mM Tris pH 8.0, 150 mM NaCl, 1 mM EDTA, and 0.5% NP-40) or Triton buffer before being resolved by SDS-PAGE.
In vitro Methylation
Recombinant GST-ULK1-IDR domain proteins purified from bacteria were incubated with immunoprecipitated ULK1 and radioactive methyl donor. The separated samples were then transferred from the gel to a PVDF membrane, which was then sprayed with EN^{3}HANCE and exposed to X-ray film.
Cell viability Assay
Two thousand cells/well were plated in 96-well plates for 24 h and then treated with IμM GSK3326595(PRMT5 inhibitor), 10μM chloroquine and 1μM MRT6921for 4 days. Fresh medium containing the drugs were replaced after 48 h. Survival cells were determined using the Cell Titer-Glo luminescent cell viability assay kit .

RESULTS



PRMT5 depletion and overexpression induces and suppress autophagy respectively. A. Immunoblot (IB) analysis of whole cell lysate (WCL) derived from MCF7 cells infected with PRMT5 sgRNA virus. **B.** Immunofluorescence analysis GFP-RFP-LC3 in MCF7 cells depleted of PRMT5. **C.** Quantification of red and yellow punctae upon PRMT5 knockout in MCF7 cells stably expressing GFP-RFP-LC3 .**D.** IB of whole cell lysates from MCF7 cells expressing wild type and Enzyme dead PRMT5.

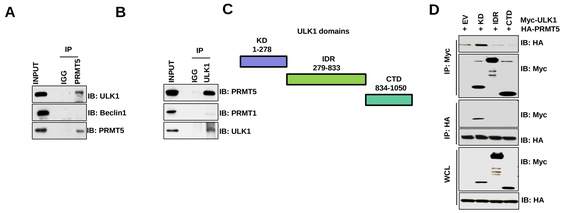


Fig. 2. ULK1 binds PRMT5 via the kinase domain ULK1. A-B. IB analysis of whole cell Lysate and immunoprecipitate (IP) derived from MCF7 and MDA-231 cells **. C.** ULK1-1 domains **D.** In vitro methylation assays were performed with GST-ULK1-IDR proteins as substrates and HA-PRMT5 as the methyltransferase.

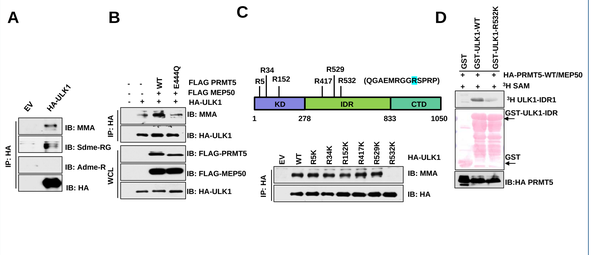


Fig. 3. PRMT5 methylates ULK1. A-B. IB analysis of WCL and immunoprecipitate (IP) derived from 293T cells transfected with indicated constructs. **C.** Predicted top-ranked arginine residues and IB analysis of IP derived from 293T cells transected with indicated ULK1 constructs. **D.** In vitro methylation assays were performed with GST-ULK1-IDR proteins as substrates and HA-PRMT5 as the methyltransferase.

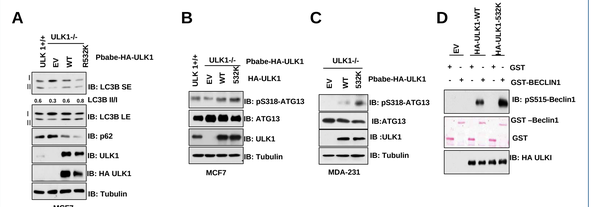


Fig.4. Methylation deficient ULK1 is hyperactive that wild type ULK1. A-C. IB analysis of WCL derived from MCF7 and MDA-231 cells expressing ULK-WT and ULK-R532K **D.** Invitro kinase assays performed from GST-Beclin-1 purified from bacteria and ULK-WT/R532K immunoprecipitated from 293T cells after transfection.

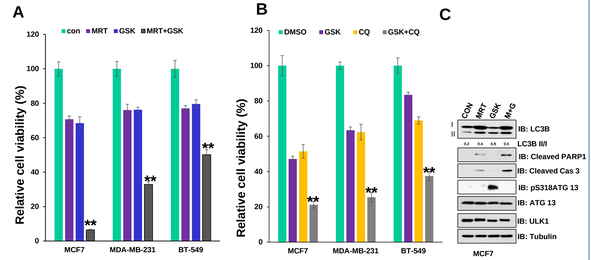


Fig. 5. PRMT5 inhibitor and autophagy inhibitors has synergistic effect. A-B. The breast cancer cells were treated with 1 μM PRMT5 inhibitor GSK3326595 (GSK), 10 μM chloroquine (CQ) or 1 μM ULK1 inhibitor MRT68921 (MRT) for 48 hrs before performing cell viability assays using the ATP-based CellTiter-Glo kit. **C**. IB analysis of WCL derived from MCF7 cells treated with indicated inhibitors for 48hrs.

CONCLUSION

- PRMT5-mediated methylation of ULK1 represses autophagy
- Methylation deficient ULK-1 is hyperactive than the wild type
- Autophagy induced by PRMT5 inhibitors appears cytoprotective
- PRMT5 inhibitors synergized with ULk1 inhibitors suppress

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