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DNA Methyltransferases Expression in Triple-negative Breast Cancer Predicts Sensitivity to Decitabine

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Introduction

Triple-negative breast cancer (TNBC) is a heterogeneous disease with poor prognosis and lacking targeted therapies especially in patients with chemotherapy resistant disease. Since DNA methylation-induced silencing of tumor suppressors is common in cancer, reversal of promoter DNA hypermethylation by decitabine (5-aza-2'-deoxycytidine), an FDA-approved DNA methyltransferase (DNMT) inhibitor, has proven effective in treating hematological neoplasms. However, its antitumor effect varies in solid tumors, stressing the importance of identifying biomarkers predictive of therapeutic response.

Methods

Breast cancer TNBC patient derived xenograft (PDX) models were used to determine correlation between DNMTs and decitabine response. Breast cancer cell lines, Hs 578T, BT-549 and MDA-MB-231 cells were used to perform in vitro experiments. To determine the decitabine effect on DNMTs, cells were treated with 100 nM of decitabine for 7 days, followed by determining DNMT levels and IP was performed to test decitabine induced DNMT ubiquitination. To understand the E3 ligase, TRAF6 effect, cells were transfected with TRAF6 siRNAs and DNMT levels, decitabine cytotoxicity as well as global DNA methylation was determined by Western blot analysis, MTS assay and blotting with anti-5-methylcytosine monoclonal antibody, respectively.

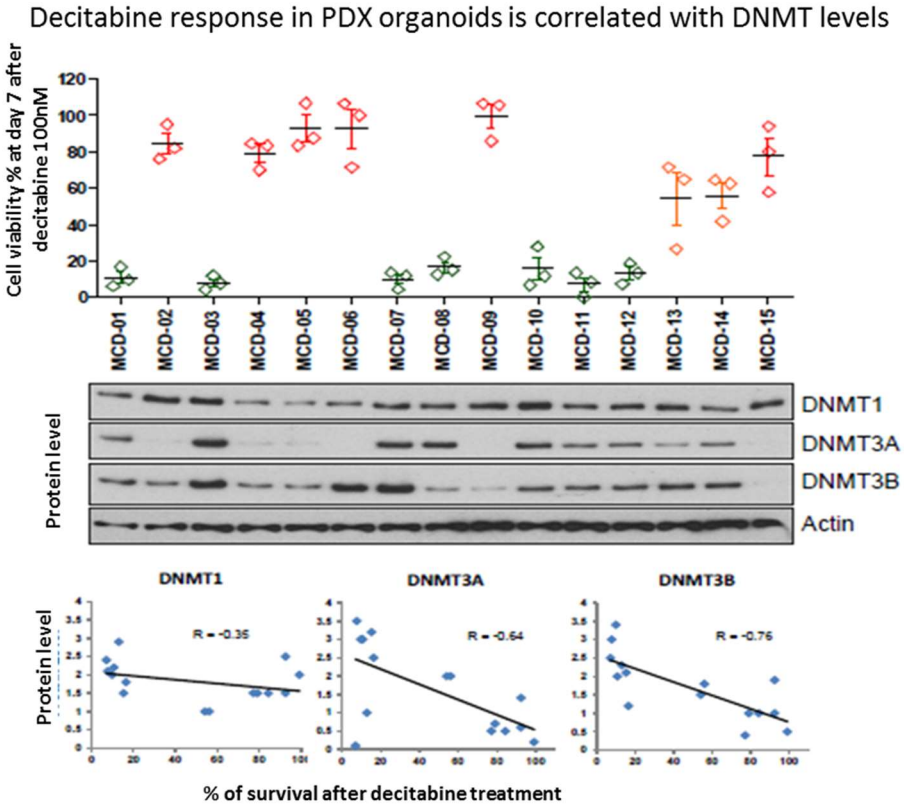
Results

We showed that protein levels of DNMTs correlated with response to decitabine in TNBC patient derived xenograft (PDX) organoids, suggesting DNMT levels as potential biomarker of response. Furthermore, all three methytransferases, DNMT1/3A/3B, were degraded following low-concentration, long-term decitabine treatment both in vitro and in vivo. The DNMT proteins could be ubiquitinated by the E3 ligase, TNF receptor associated factor 6 (TRAF6), leading to lysosome-dependent degradation. Depletion of TRAF6 blocked decitabine-induced DNMT degradation, conferring resistance to decitabine.

Discussion

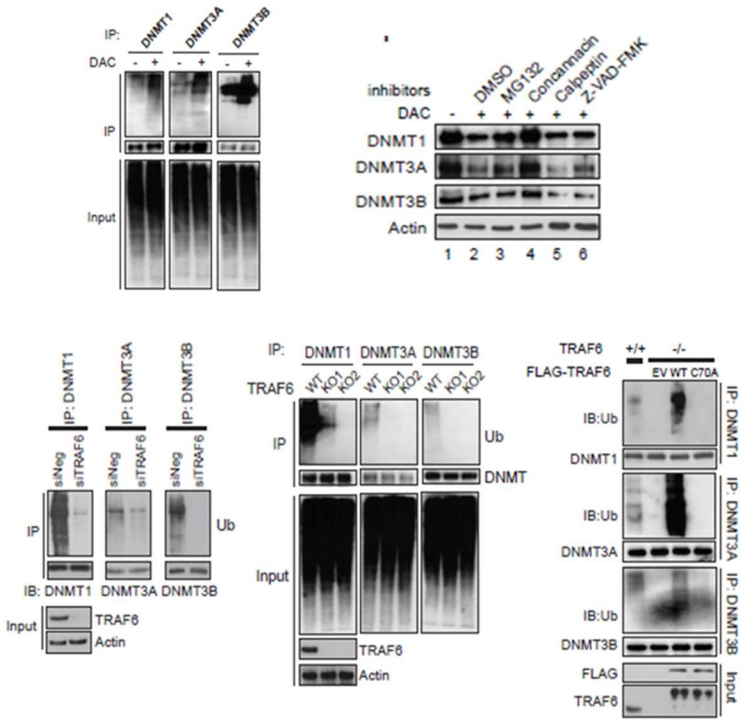
Our study suggests that decitabine induces degradation of DNMT1, DNMT3A and DNMT3B by TRAF6 through a lysosome dependent degradation pathway. This is one major mechanism by which decitabine inhibits tumor growth. DNMT protein levels might serve as a potential biomarker to guide the drug selection. Finally, TNBC PDX responded to decitabine regardless of chemotherapy response. Therefore, TNBC patients with high DNMT levels and resistance to standard chemotherapy may still benefit from decitabine. Future clinical studies are required development to test decitabine like drugs in high risk TNBC patients.

Slide 1:



Slide 2:

Decitabine effect on DNMT protein levels is regulated through lysosome dependent degradation pathway through the E3 ligase, TRAF6



Slide 3:

Knocking Down TRAF6 induced DNMT protein levels and desensitize cells to decitabine, indicating its essential role in decitabine action