DNA Protein Kinase (DNA-PKcs) mediated transcriptional regulation of TOP2ß drives chemoresistance in leukemia

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High mortality in AML is due to acquired chemo-resistance and relapse emphasizing the need for better therapeutic strategies. To understand the molecular basis of acquired resistance in AML we developed clinically relevant cellular models that recapitulate acquired chemoresistance in clinics. Using these model systems and patient samples, we demonstrate that unlike in sensitive parent cells, in relapse cells DNA damaging drugs (anthracyclines) fail to induce DNA double strand breaks despite similar drug uptake. Accordingly, there was no gamma H2AX or differential expression of DDR proteins Ku80, Mre11, Nbs1, Rad50, and ATM. However, compared to sensitive cells, DNA repair kinase protein- DNA-PKcs was significantly overexpressed at mRNA and protein levels in the relapse cells. DNA-PKcs was also found to be overexpressed in 2 independent set of relapse patient samples (n=50 and n=20) and was significantly associated with poor prognosis. Pharmacological or genetic knockdown of DNA-PKcs rendered refractory relapse cells sensitive to anthracyclines and induced complete apoptosis in these cells. Mechanistically, knockdown of DNA-PKcs in resistant relapse cells re-expressed TOP2B (anthracycline target) making these cells sensitive to anthracyclines. ChIP-qPCR and luciferase promoter assays with multiple deletion constructs of TOP2B promoter region showed that DNA-PKcs binds to the region between 550bp and 440bp upstream to the transcription start site of TOP2B gene and acts as a co-repressor of TOP2B gene expression. Analysis of single-cell transcriptome data from 96 clones (dbgap) showed significant inverse correlation between the expression of DNA-PKcs and TOP2B. Accordingly, knockdown of TOP2B rescued the DNAPKcs knockdown phenotype. Additionally, we demonstrate that increased DNA-PKcs expression in relapse cells is due to GCN5 (acetyltransferase) mediated acetylation of H3K27 residues in the promoter of DNAPKcs and knockdown of GCN5 downregulated DNA-PKcs expression.

Taken together, we identify a non canonical role of DNA-PKcs, a kinase central to the DNA damage response in mediating acquired resistance to anthracyclines in AML by acting as a transcriptional repressor of anthracyclin target gene-TOP2B. We also demonstrate DNA-PKcs as a novel therapeutic target for refractory relapse cells.