Novel roles of NUDT15 in antileukemic therapies revealed by combining phenotypic screening and pharmacological inhibition

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Cancer cells disrupt and exploit host nucleotide metabolism to fuel their rapid proliferation and heightened DNA repair needs, and thereby are particularly vulnerable to nucleotide imbalance and/or cytotoxic/mutagenic nucleotide analogue (NA) drugs. NUDIX-type 15 (NUDT15) is a nucleotide pyrophosphatase against canonical nucleotides as well as the metabolites of multiple NA drugs (e.g. thiopurine, ganciclovir and acyclovir). Whilst previous research by us and others have established NUDT15 could be a targetable biomarker dictating therapeutic outcome of NA drugs through direct hydrolysis and inactivation, however, it has yet to addressed if NUDT15 could indirectly influence non-NA oncology drug efficacy by inducing nucleotide imbalance.

Here we unbiasedly profiled the role(s) of NUDT15 in both NA and non-NA oncology drug activities, by combining our recently published *bona fide* NUDT15 probes with a pharmacogenetic screening platform utilizing acute myeloid leukemia cell lines. From the NA drug screening study, we identified that NUDT15 is a key metabolizer and resistance factor of 3-deazauridine (DAU), a CTP synthase inhibitor clinically tested against acute leukemia. NUDT15 ablation in leukemic HL-60 and NB4 cells aggravated DAU-induced replication stress and thereafter apoptosis, which, critically, were phenocopied by two distinct nanomolar orthosteric NUDT15 inhibitors, TH8321 and TH7755, in a panel of leukemic cell lines. Building upon the critical role of NUDT15 in DAU efficacy, synthetic lethality between DAU and clinically relevant Chk1/Wee1 inhibitors was further established, particularly in leukemic cells of NUDT15-null background.

Concurrently, we employed the NUDT15 pharmacogenetic screening platform against a FDA-Approved Oncology Drug Set library (NCI) and unexpectedly uncovered that NUDT15 has a ‘gate-keeper’ role for AMPL differentiation therapy, i.e. tretinoin and calcitriol. NUDT15 level negatively correlated with tretinoin efficacies in APML cell lines, and ablation drastically reduced drug EC50 by 1000-fold in HL-60 cells through promoting terminal differentiation.

Overall, study presented herein highlighted NUDT15 as a clinically relevant target and marker for a broad spectrum of oncology drugs, potentially indicating new avenues in antileukemic therapies.

Keywords: nucleos(t)ide analogue antimetabolite, nucleotide metabolism, AML, differentiation