Systematic profiling of drug metabolite interactions with the dNTPase SAMHD1

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Objectives: Our lab recently identified the deoxynucleoside triphosphohydrolase (dNTPase) SAMHD1 as a resistance factor to Cytarabine therapy in leukaemia. Subsequently, other triphosphate metabolites of nucleoside analogues have been identified as SAMHD1 substrates and even allosteric activators. However, a complete understanding of which antimetabolite chemotherapies are controlled by SAMHD1 is currently lacking. We therefore aim to conduct the first comprehensive small molecule interactor screen to examine the allosteric and catalytic interactions between SAMHD1 and antimetabolite cancer drugs.

Methods: The interactor screen was performed using a high-throughput enzyme-coupled activity assay, where nucleotide analogues were combined with known activators or substrates of SAMHD1 to differentiate between allosteric and active-site interactions. Follow-up studies were performed using a pipeline of biochemical and biophysical assays. This includes enzyme activity assays to compare analogues to their endogenous nucleotide counterparts (high-throughput endpoint, continuous kinetics), a competitive binding assay to study allosteric site affinity, as well as assays to study SAMHD1 oligomerisation (chemical crosslinking, mass photometry).

Results: The comprehensive interactor screen successfully identified novel interactions between SAMHD1 and nucleoside analogue metabolites. This includes metabolites of clinically relevant chemotherapy drugs Fluorouracil and Thioguanine. We further characterised the screen hits using biophysical and biochemical methods, showing that some analogues allosterically activate their own hydrolysis by promoting SAMHD1 oligomerisation and are more readily hydrolysed than their endogenous counterparts.

Conclusions: We have identified novel interactions between chemoresistance factor SAMHD1 and metabolites of clinically relevant antimetabolite cancer drugs, both as substrates and as allosteric enzyme activators. The diverse range of chemical modifications present in the screened nucleotide analogues also enables us to map out individual base or sugar modifications and their effect on allosteric or active site binding, allowing the prediction of interactions with compounds not included in the screen. In addition, the comprehensive characterisation of SAMHD1 interactors will allow for better-informed treatment options based on the SAMHD1 status in cancer patients.

Relevant references:

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