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# **Roles of NUDT5 and NUDT15 beyond oxidized nucleotide metabolism and their potential as therapeutic targets**

**AKADEMISK AVHANDLING**

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av

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## ABSTRACT

The nucleotide precursor pool is readily susceptible to numerous sources of modification and damage, including alkylation, deamination and oxidation/nitrosylation, among others; most of which have deleterious effects on nucleic acid integrity and cellular fitness. In addition to DNA repair mechanisms, these metabolic byproducts are kept in check by so-called sanitation or “housekeeping” enzymes, chief among them the NUDIX hydrolase superfamily. Increased metabolic demand and strain in certain contexts, such as cancer, may require a greater reliance on these proteins; therefore, they are attractive drug targets.

The human NUDIX hydrolase, MTH1 (NUDT1), sanitizes the nucleotide pool of 8-oxo-guanine triphosphates, considered the most common oxidative lesion, thereby preventing mutagenesis of nucleic acids and preserving their integrity. Other NUDIX enzymes, namely NUDT15 (MTH2) and NUDT5, are proposed to perform similar functions as MTH1, and, therefore, may serve as resistance mechanisms for cells treated with MTH1 inhibitors. However, very little is known about their biological functions in human cells.

The focus of this thesis was to determine the biological roles of NUDT15 and NUDT5 and if they are desirable drug targets for treating cancer. Surprisingly, we found that neither of these proteins appeared to be important for oxidized nucleotide metabolism, but, rather, they had unexpected and diverse functions in nucleotide metabolism with cancer therapeutic implications. These findings should encourage further study of the human NUDIX family.

In **Paper I**, we compared NUDT15 biochemically, structurally and in a cellular context to MTH1. NUDT15 hydrolyzed 8-oxo-dGTP about 230-fold less efficiently than MTH1, and its depletion in cancer cells neither affected cell survival nor oxidized nucleotide content of DNA. The NUDT15 crystal structure explained this deviation from MTH1 and shows that 8-oxo-dGTP is poorly accommodated in the enzyme active site. We also identified 6-thio-(d)GTP, the active metabolites of thiopurine chemotherapeutics, as NUDT15 substrates.

In **Paper II**, we expounded upon the role of NUDT15 in thiopurine metabolism and why the R139C missense mutant causes thiopurine intolerance in patients. NUDT15 efficiently hydrolyzes 6-thio-(d)GTP, thus mediating the amount of the active thiopurine metabolites in cells. In addition, the R139C mutation does not impact catalytic ability of NUDT15, but rather causes destabilization of the protein structure and proteolytic degradation in cells, thus explaining why patients with this mutation are sensitive to thiopurine treatments.

**Paper III** presents further evidence that NUDT5 may not be an important contributor to sanitation of the oxidized nucleotide pool, describes the first small molecule NUDT5 inhibitors and confirms the nuclear ATP synthetic role for NUDT5 in breast cancer cells. Following an initial screening campaign and medicinal chemistry efforts, potent, cell-active NUDT5 inhibitors were identified using a CETSA-guided screening funnel. Lead compound, TH5427, abrogated progestin-dependent gene regulation and proliferation in breast cancer cells, thus, representing a bonafide probe to further study NUDT5 biology.