The Structural, Mechanistic, and Clinical Applications of NT5C2 in Drug Resistance and Relapse of T-ALL

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Genetic mutations in patients with relapsed acute lymphoblastic leukemia (T-ALL) cause the NT5C2 enzyme to act as if it has been allosterically activated, allowing purine nucleoside substrates and purine analog chemotherapy drugs such as 6-Mercaptopurine (6-MP) to be continuously processed in the catalytic site. This causes drug inhibition by the NT5C2 enzyme, resulting in chemotherapy resistance. In this study, the structural abnormalities of NT5C2 mutations determined through previous research were exploited using various potential inhibitors designed to reduce enzyme activity. CRISPR / Cas9 gene editing technique was used to engineer mutations in the NT5C2 loop to determine which areas of the enzyme complex are responsible for negative feedback mechanisms. Furthermore, the novel pharmaceutical drug, Mizoribine, was tested in comparison to 6-MP to determine its ability to target only mutant NT5C2 cells while sparing WT cells. It was found that of 40 compounds tested, compounds 11 and 20 had the greatest inhibitory effects due to the phosodiester bonds they contain as ribonucleoside phosphates. The deletion of R413-D415 in the loop region caused increased de-regulation of NT5C2 and therefore more chemotherapy resistance, suggesting that longer loops will act as better negative feedback mechanisms. Lastly, Mizoribine worked better than 6-MP as a treatment against mutant NT5C2 because it depletes the already stressed de novo biosynthesis pathway of purines. Future research aims to up-regulate loop activity by engineering insertion mutations and to test 6-MP with compounds 11 and 20. These findings provide structural, mechanistic, and clinical applications towards the treatment and prevention of relapsed T-ALL.

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Second Award of $2,000