**Investigation of Mutations Associated with 5-Fluorouracil Drug Resistance in Colorectal Cancer Cell Line HCT116**

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Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths worldwide. Standard treatment options include surgery, radiation therapy, and chemotherapy. Among chemotherapeutic agents, 5-fluorouracil (5-FU) has long been a cornerstone in CRC treatment. 5-FU primarily acts by inhibiting thymidylate synthase (TS), thereby interfering with DNA synthesis and suppressing tumor cell proliferation. Despite its widespread use, resistance to 5-FU remains a significant clinical challenge, often leading to therapeutic failure and poor patient outcomes.

Previous studies have demonstrated that 5-FU resistance can arise through multiple mechanisms, including overexpression of TS, loss of function in tumor suppressor genes (TSGs), altered drug transport, and increased activity of dihydropyrimidine dehydrogenase (DPD). In this study, we investigated the genetic basis of 5-FU resistance using the HCT116 colorectal cancer cell line. We established cell lines with varying degrees of resistance to 5-FU and conducted next-generation sequencing (NGS) to identify potential resistance-associated mutations. Among several candidates, a point mutation in a specific gene was found to be strongly associated with increased resistance.

To further explore the structural implications of this mutation, we will utilize AlphaFold, an AI-based protein structure prediction tool, to model and compare the three-dimensional structures of the wild-type and mutant proteins. This will help assess whether the mutation induces significant conformational changes that may underlie the resistance phenotype. In parallel, we plan to conduct gene knockout experiments in the drug-resistant cell lines to evaluate the functional role of this gene in mediating 5-FU resistance, thereby providing deeper insights into its potential as a therapeutic target.