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Effect of Uracil DNA Glycosylase Activity on the Efficacy of Thymidylate Synthase Inhibitor/HDAC Inhibitor Combination Therapies in Colon Cancer

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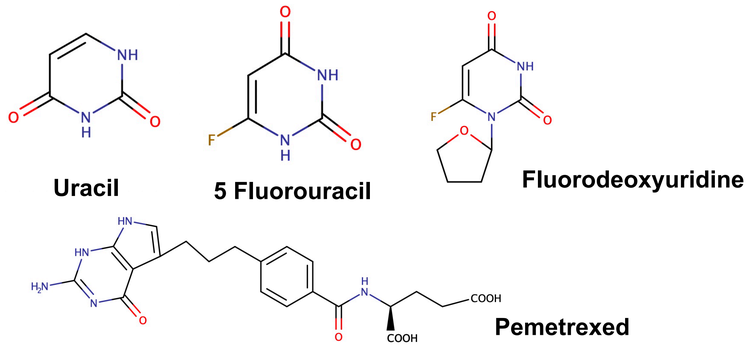
Abstract

Human uracil DNA glycosylase (UNG2) is responsible for removing uracil bases from DNA and initiates base excision repair pathways. Accumulation of uracil or its fluorinated analogs in DNA is one of the killing mechanisms of thymidylate synthase (TS) inhibitors in cancer cells, and depletion of UNG2 often enhances the toxicity of these anticancer drugs. We tested the effect of UNG2 KO on the efficacy of multiple TS inhibitors (5-fluorouracil, fluorodeoxyuridine, and pemetrexed), and we determined that except for 5-fluorouracil, all other TS inhibitors were significantly more potent in UNG2 KO cells compared to wild-type HT29 cells. Interestingly, UNG2 protein levels can also be depleted by the HDAC inhibitors SAHA and MS275, providing a pharmacologic strategy to reduce UNG2 activity in cells. Unexpectedly, the HDAC inhibitors synergized with 5-fluorouracil but not fluorodeoxyuridine in both wild-type and UNG2-knockout cells. Similarly, HDAC inhibitors synergized with pemetrexed in wild-type HT29 but not UNG2-knockout cells. This suggested that HDAC inhibitors sensitized cells to 5-fluorouracil through an UNG2-independent mechanism. Interestingly, SAHA depleted the UNG2 level, whereas TS inhibitors alone and their combination with SAHA upregulated the level of UNG2 at 24 hours. This suggests HDAC inhibitors deplete UNG2, but when combined with TS inhibitors, it did not affect UNG2, at least at a concentration of 100nM. Our future aim is to study these pharmacological drug combinations targeting UNG2 activity in cells and elucidate exact mechanisms of cell death.

Introduction

- Human uracil DNA glycosylase (UNG2) removes uracil bases from DNA. UNG2 depletion in cancer cells is associated with increased genomic uracil leading to DNA damage and cell death and is an anticancer mechanism of thymidylate synthase[1].
- Thymidylate synthase (TS) catalyzes the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP) [1].
- TS inhibitors include 5FU, FDU, and pemetrexed



- Literature suggests that HDAC inhibitors pharmacologically deplete UNG2 and TS level[2-4].

Results

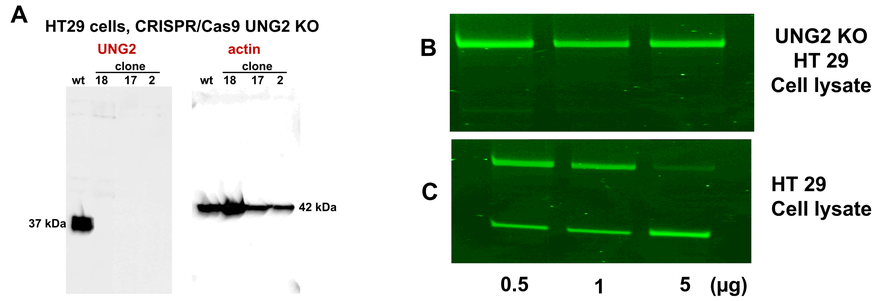


Fig 1: Crispr/Cas9 knockout of UNG2 in HT29. Western blots for UNG2 and actin were performed on UNG2 KO clones and wild-type HT 29 cell lysates (A) UNG2 activity assay confirming knock out of UNG2 gene in (B) UNG2 KO cells compared to (C) wild-type HT 29 cells.

Cell Viability Assay at 72 h

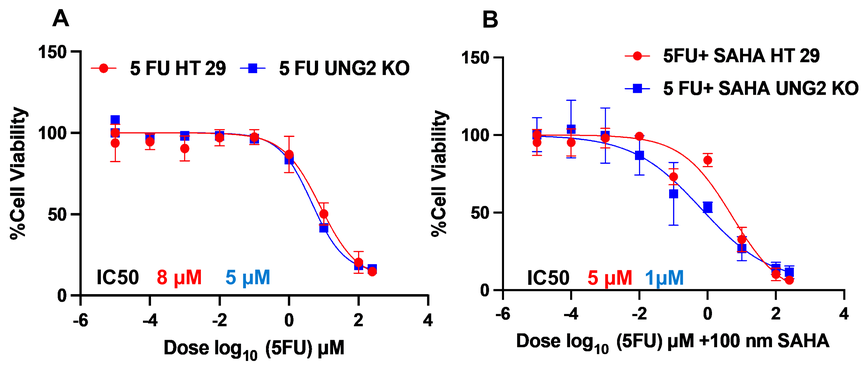


Fig 2: Effect of 5FU on cell viability in HT29 and UNG2 KO. Cells were treated with different concentrations of 5FU alone (A) and in combination with SAHA (B) for 72 h and MTT was performed.

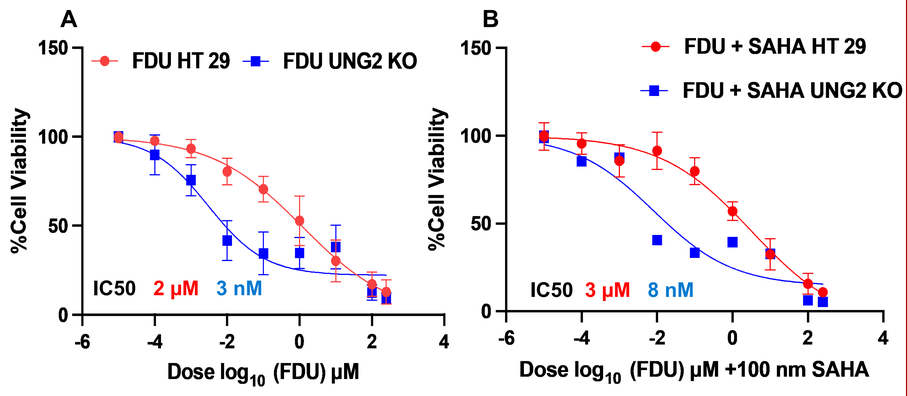


Fig 3: Effect of FDU on cell viability in HT29 and UNG2 KO. Cells were treated with different concentrations of FDU alone (A) and in combination with SAHA (B) for 72 h and MTT assay was performed.

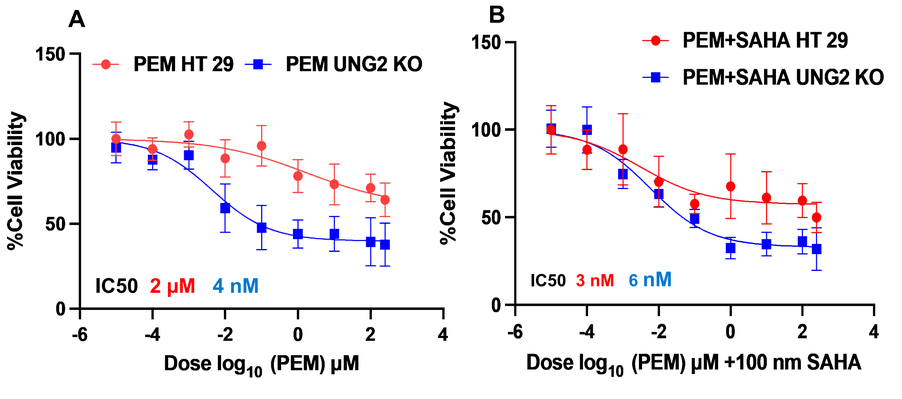


Fig 4: Effect of Pemetrexed on cell viability in HT29 and UNG2 KO. Cells were treated with different concentrations of pemetrexed alone (A) and in combination with SAHA (B) for 72 h and MTT assay was performed.

UNG2 Expression at 24 h

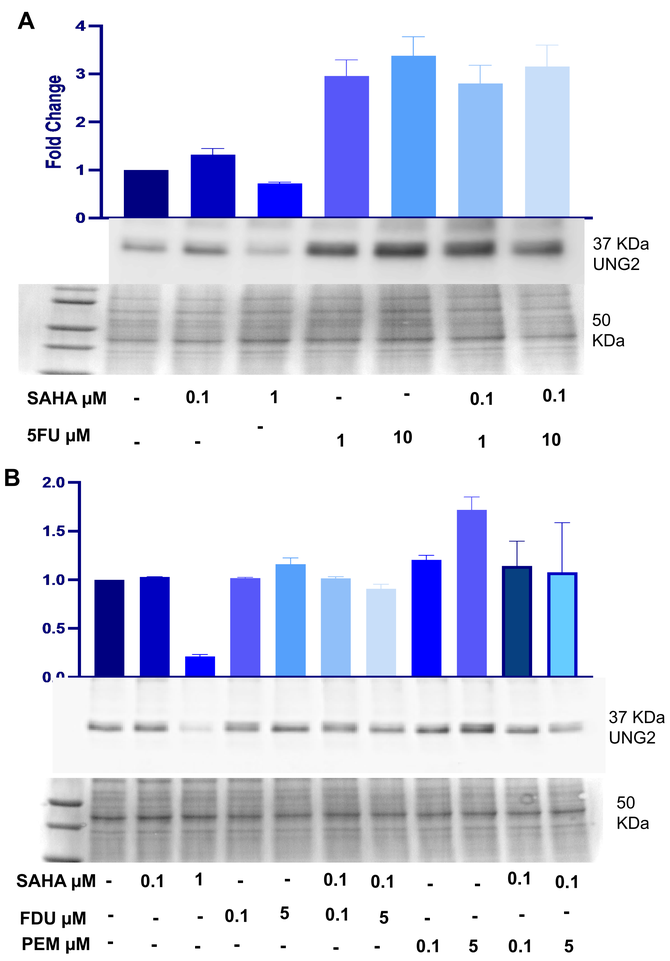


Fig 5: UNG2 expression by western blot. HT29 cells were treated with SAHA and 5FU at the above concentrations (A) and with FDU, PEM at above concentration (B) Cell lysates were prepared after 24 h and western blot was performed.

Summary

- FDU and Pemetrexed were significantly more potent in UNG2 KO cells compared to wild-type HT29 cells.
- HDAC inhibitors synergized with 5-fluorouracil, but not fluorodeoxyuridine, in both wild-type and UNG2-knockout cells.
- Pemetrexed synergized with SAHA only in wild type but not in UNG2 knockout cells.
- SAHA down regulates UNG2 expression at high, but not low concentrations.

Acknowledgement

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References

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