

## Uracil DNA Metabolism as a Target for Chemotherapy

### Background

Antifolates and fluoropyrimidines have been widely used as antineoplastic agents in the treatment of leukemias, head and neck, breast and gastrointestinal cancers. These chemotherapeutic agents target key enzymes of thymidylate metabolism. Chemotherapeutic agents such as 5-fluorouracil (5FU) and fluorodeoxyuridine (FdUR) inhibit thymidylate synthase enzyme directly leading to a block in the de novo biosynthesis of thymidine monophosphate (TMP) from the precursor dUMP. TMP is generated when dUMP accepts a methyl group from 5,10 methylenetetrahydrofolate (MTHF). Antineoplastic agents such as aminopterin inhibit the enzyme dihydrofolate reductase (DHFR), a critical enzyme in the pathway leading to the generation of MTHF, thereby blocking the generation of TMP by limiting the availability of the methyl donor.

It is widely accepted that the depletion of TTP pool is the primary mechanism by which inhibitors of thymidylate synthase and DHFR mediate tumor killing. This process termed “thymineless death” is due to an arrest in DNA synthesis and DNA degradation. Recent studies have provided evidence that there are other mechanisms such as misincorporation of Uracil into DNA or an imbalance in other dNTP pool that can contribute to the conditions of thymineless death. Uracil DNA glycosylase (UDG) and dUTPase are two critical enzymes that are involved in the removal (excision repair) of misincorporated uracil in DNA and regulating the available dUTP pool, respectively. **The current invention reveals the possibility of exploiting these enzymes as additional targets for the development of chemotherapeutic agents.**

### Description of the Technology

Cells overexpressing dUTPase, UDG, and uracil-DNA glycosylase inhibitor (Ugi) of bacteriophage PBS2 origin, and uracil-DNA glycosylase deletion mutants (*ung1*) were generated as model systems for the investigation of aberrant uracil-DNA metabolism. The strain overexpressing the enzyme dUTPase prevents misincorporation of uracil into DNA in the presence of antifolate and also has severely reduced TTP and dUTP levels. Compared to controls that arrest in S phase, which is indicative of uracil misincorporation, the UTPase strain demonstrates cell cycle arrest in mid S-phase suggesting an imbalance in dNTP pool. An examination of the dNTP pool confirmed lack of thymidine or uracil. UTPase overexpression also confers greater resistance to antifolate compared to Ugi or UDG expressing strains confirming that the protective effect of overexpression of UTPase is due to lack of dUTP accumulation.

Compared to UTPase strains, cells that overexpress Ugi have uracil misincorporation in DNA in the presence of antifolate and arrest in G2/M boundary. These strains also demonstrate complete lack of replication intermediates, a condition that is consistent with stable uracil misincorporation. By contrast, the UTPase and UDG strains had elevated replication intermediates consistent with the existence of abandoned replication complexes due to lack of deoxy nucleotides for replication.

The *ung1* deletion mutants bypass the S-phase arrest and have stably misincorporated uracil and were able to complete replication. However these mutants were lethal at later time points. Strains expressing UDG do not exhibit greater lethality. Taken together, these data suggest a definitive role for impaired uracil-DNA metabolism in thymineless death and that TTP pool depletion is not the only mechanism of antifolate induced cytotoxicity. These findings provide additional targets that can be exploited for the development of chemotherapeutic agents.

### **Advantages**

- Provides a way to estimate the effectiveness of chemotherapy in cancer patients and to individualize dosage and treatment methods.
- Specificity: UDG and dUTPase are specific to uracil enabling identification of agents that modulate uracil misincorporation without false positives.

### **Applications**

- For screening agents capable of promoting or reducing uracil misincorporation into DNA
- For screening agents capable of inhibiting dUTPase and UDG for the purpose of developing novel cytotoxic agents.
- Kits having UDG, UTPase and Ugi overexpressing strains for developing assays for uracil misincorporation
- To determine effectiveness of chemotherapy by quantifying uracil misincorporation in DNA
- For the developing anti-infectives
- As research tools

### **Deliverables**

- Yeast strains overexpressing dUTPase, uracil DNA glycosylase, uracil-DNA glycosylase inhibitor (Ugi) of bacteriophage PBS2 origin and uracil DNA glycosylase (*ung1*) deletion mutants
- An enzyme-based assay for the determination of uracil misincorporation and replication intermediates in the presence of antifolate has been developed.
- An assay for the determination of dUTP and TTP pool sizes
- Primers, expression vectors for dUTPase, uracil DNA glycosylase, *ung1* deletion mutants and Ugi, and sequences

### **Patent Status**

- United States patent application filed.
- Application was published on June 27, 2002 (Publication No.: US-2002-0081594 A1)

### **Licensing Opportunity**

- This technology will be licensed non-exclusively as a research tool and exclusively for therapeutic uses.

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