



Citation for published version:

Hussain, S 2020, 'On a new proposed mechanism of 5-fluorouracil-mediated cytotoxicity', *Trends in Cancer*, vol. 6, no. 5, pp. 365-368. <https://doi.org/10.1016/j.trecan.2020.02.009>

DOI:

[10.1016/j.trecan.2020.02.009](https://doi.org/10.1016/j.trecan.2020.02.009)

Publication date:

2020

Document Version

Peer reviewed version

[Link to publication](#)

Publisher Rights

CC BY-NC-ND

University of Bath

Alternative formats

If you require this document in an alternative format, please contact:
openaccess@bath.ac.uk

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

1

2

3

4

5

On a new proposed mechanism of 5- fluorouracil-mediated cytotoxicity

6

7

Shobbir Hussain

8

9

Department of Biology and Biochemistry, University of Bath, Claverton Down,

10

Bath BA2 7AY, United Kingdom

11

12

13

14

Keywords: 5FU, fluorouracil, TRMT2A, NUD1, m5U, 5-methyluridine, RNA

15

methylation, DNA break repair, homologous recombination

16

17

Correspondence: s.hussain@bath.ac.uk

18

19 **Abstract**

20 The major molecular mode of action of the cytotoxic drug 5FU is generally considered to
21 result from thymidylate synthase inhibition. Recent findings relating to the function of the
22 human uracil-5 methyltransferase, TRMT2A, and its interaction with 5FU metabolites
23 incorporated within tRNAs, lead to an additional hypothesis which is proposed here.

24

25

26

27

28

29

30

31

32

33

34

35

36

37

38

39

40

41

42 The synthetic uracil analogue 5-fluorouracil (5FU) is an important chemotherapeutic drug
43 with a cytotoxic effect associated with an increased susceptibility to DNA breaks [1, 2]. The
44 compound is converted to several active metabolites within cells including the nucleotides
45 fluoro-deoxyuridine-monophosphate (FdUMP), fluoro-deoxyuridine-triphosphate (FdUTP),
46 and fluoro-uridine-triphosphate (FUTP). The mechanism of action of 5FU and its cellular
47 derivatives have been widely studied, and an inhibition of the deoxyuridine-monophosphate
48 (dUMP) to deoxythymidine-monophosphate (dTMP or 'thymidylate') conversion enzyme,
49 thymidylate synthase, is generally considered to be the major route toward cytotoxicity [1].
50 FdUMP binds irreversibly to the nucleotide-binding pocket of thymidylate synthase thus
51 inhibiting dUMP to dTMP conversion. Although dTMP can be readily salvaged from
52 thymidine via action of the thymidine kinase enzyme, thymidylate synthase inhibition-
53 induced nucleotide imbalances can still lead to some misincorporation of deoxyuridine-
54 triphosphate (dUTP) and FdUTP into DNA. The rectification of such misincorporation can
55 usually be mediated by DNA repair excision enzymes for example, however, futile rounds of
56 misincorporation-excision repair may ensue which might potentially lead to the formation
57 of DNA breaks and thus cell death [1]. The actual details of thymidylate synthase inhibition-
58 induced nucleotide misincorporation in the induction of DNA breaks and their inefficient
59 repair however remain poorly characterised [1, 2]. Parallel observations that 5FU treatment
60 leads to impaired repair of DNA breaks by homologous recombination [3], are nonetheless
61 worth noting [2].

62 Also to be considered, is the fact that the FUTP nucleotide is extensively incorporated into
63 RNA in direct competition with the natural uridine-triphosphate (UTP) nucleotide during
64 gene transcription. The idea that this may contribute to the cytotoxic effect of 5FU is
65 supported by the observation of a strong correlation between FUTP incorporation into RNA
66 and attenuation of clonogenic survival in multiple cancer cell lines [4, 5]. Although
67 misregulated pre-rRNA processing and pre-mRNA splicing events for example have since
68 been associated with the presence of FUTP in RNA, whether any of these play significant
69 roles in 5FU-induced cytotoxicity remains to be elucidated [1].

70 A recent study by Carter et al. has demonstrated that the human uracil-5 methyltransferase
71 (U5MT), TRMT2A, targets uridine-54 of tRNAs as a substrate for methylation [6]. The
72 investigations involved revealing that cellular 5FU exposure led to extensive and irreversible

73 crosslinking between the TRMT2A enzyme and target ribonucleotides within tRNAs. The
74 biochemical mechanism of the relevant methylation reaction was indeed previously known
75 to occur via an enzyme-substrate covalently crosslinked complex [7]. Normally, such a
76 complex is only a transient intermediate in the catalytic reaction and is accordingly quickly
77 resolved thus releasing the methylated RNA and regenerating the methyltransferase [7]
78 (illustrated schematically in Figure 1A). However, the incorporation of FUTP into U5MT
79 target RNA sites leads to the enzyme becoming permanently crosslinked to the analogue
80 ribonucleotide during the methylation reaction [6, 7] (Figure 1B). This principle was used to
81 map the precise enzymatic target sites of TRMT2A in cellular RNAs by Carter et al., but the
82 observations also raise the question of what biological effect the relevant unnatural
83 irreversible crosslinking might have within the cell.

84 Although TRMT2A was found to target numerous tRNA isoacceptors [6], as the cellular pool
85 of each isoacceptor is significantly larger than the number of cellular TRMT2A molecules,
86 the deleterious effect of 5FU-mediated TRMT2A-tRNA crosslinking on overall tRNA function
87 is probably quite limited. Nonetheless, what about the potential effects of TRMT2A function
88 inhibition? This is a particularly relevant question to ask as we know of prominent parallel
89 examples where cellular exposure to the cytidine analogue, 5-azacytidine, leads to
90 permanent crosslinking of DNA cytosine-5 methyltransferases to DNA (in fact via a similar
91 enzymatic crosslinking mechanism as observed for TRMT2A-RNA), thus leading to
92 functionally relevant depletion/inhibition of the DNA methylation enzymes. Could an
93 analogous 5FU-mediated TRMT2A inhibition lead to a significant cytotoxic effect? If we're
94 considering just cellular loss of the methyltransferase functions of the enzyme, then
95 probably not, as eukaryotic cells lacking tRNA uridine-54 methylation exhibit only very mild
96 observable phenotypes [8]. It is intriguing however that while inactivation of the
97 methyltransferase activity of the bacterial homolog of TRMT2A, TrmA, only leads to mild
98 effects too, full disruption of the TrmA gene is lethal [9].

99 Indeed, while the apparently non-essential uridine-54 methylation in tRNAs might help
100 improve rates of tRNA structural folding [10], circumstantial evidence indicates that the
101 yeast homologue of TRMT2A, an alias of which includes NUD1, may have crucial orthogonal
102 cellular roles, namely in homologous recombination-mediated DNA break repair [11-13].
103 Notably, such studies demonstrated NUD1 to harbour a DNA endo-exonuclease enzymatic

104 activity which might potentially participate in an early step of the homology-directed repair
105 process [11, 12]. Although ectopic expression of NUD1 in mammalian cells was found to
106 promote extrachromosomal homologous recombination processes [12], a role for TRMT2A
107 itself in homology-directed DNA break repair however remains under-investigated and
108 currently only speculative. Nonetheless, the recent independent characterisations that
109 TRMT2A is a predominantly nuclear protein, and has a cell-cycle regulated expression profile
110 which peaks in S-phase [14, 15], should help further support studies into its roles in DNA
111 break repair. Certainly, there is no obvious reason as to why the expression of a tRNA
112 methyltransferase, whose respective activity is to promote tRNA maturation, should peak in
113 S-phase, although an additional role in homology-directed DNA repair for example would
114 offer adequate explanation. A relevant important role in homologous recombination repair
115 would also be further attractive overall, as it could explain lethality associated with TRMT2A
116 homolog deletion being observed currently only in bacteria [9], where alternative
117 compensatory routes of DNA break repair are much more limited compared to that present
118 in eukaryotic cells.

119 When considering potential links between TRMT2A and 5FU-mediated cytotoxicity, both
120 cellular and biochemical assays assessing the direct roles of TRMT2A in homology-directed
121 DNA break repair, and the effects of its disruption/inhibition in sensitising cells to DNA
122 breaks, should be a focus of further investigation. As the yeast studies on NUD1 function
123 have suggested that it might play an early role in the homologous recombination repair
124 process, this may guide efforts in elucidating any potential relevant roles for TRMT2A.
125 Should the involvement of TRMT2A in homology-directed DNA break repair be positively
126 established, then TRMT2A inhibition could well emerge as a major mechanism of 5FU-
127 mediated cytotoxicity (Figure 2).

128

129 **Figure legends**

130 *Figure 1. Schematic representations of the tRNA uracil-5 methylation reaction. (a) A target*
131 *uridine nucleotide within tRNA is the normal substrate of a U5MT enzyme (such as TRMT2A*
132 *for example) (step 1). During the catalytic reaction, the U5MT becomes covalently*
133 *crosslinked to the carbon at position 6 of the uracil ring (step 2). This results in the carbon at*

134 position 5 becoming susceptible to electrophilic attack, and a methyl group can be
135 conjugated to it (step 3). U5MT-dependent proton abstraction from the 5-carbon occurs
136 next (step 4), before release of the U5MT from the 6-carbon can occur (step 5). (b) When a
137 fluoro-uridine nucleotide is present at the target position of the tRNA, U5MT-tRNA covalent
138 crosslinking (step 2) and methyl group conjugation (step 3) both proceed normally, but the
139 U5MT cannot mediate fluoride abstraction from the 5-carbon, and thus U5MT release from
140 the 6-carbon is also blocked.

141 *Figure 2. Hypothesised TRMT2A-inhibition route of 5FU-mediated cytotoxicity.* The depicted
142 events take place in the cell nucleus, where tRNA uridine-54 methylation, an early step in
143 tRNA maturation, is known to occur. (a) Under normal conditions, the enzymatic interaction
144 of TRMT2A with tRNAs is only very transient, thus maintaining a functional pool of TRMT2A
145 in the nucleus able to participate in the repair of DNA breaks. (b) Under 5FU conditions,
146 permanent crosslinks form extensively between TRMT2A and tRNAs molecules, thus almost
147 certainly rendering the complexes non-functional. As cellular tRNA pools are large compared
148 to the TRMT2A pool, the remaining un-crosslinked tRNAs are likely sufficient to maintain
149 normal cellular protein synthesis. However, the severely depleted functional TRMT2A pool is
150 not able to maintain normal DNA break repair, which can lead to cell death. The overall
151 proposal will likely hinge on establishing the extent to which TRMT2A plays direct roles in
152 the repair of DNA breaks.

153

154 **References**

- 155 1. Longley, D.B. et al. (2003) 5-fluorouracil: mechanisms of action and clinical strategies. *Nat Rev*
156 *Cancer* 3 (5), 330-8.
- 157 2. Wyatt, M.D. and Wilson, D.M., 3rd (2009) Participation of DNA repair in the response to 5-
158 fluorouracil. *Cell Mol Life Sci* 66 (5), 788-99.
- 159 3. Srinivas, U.S. et al. (2015) 5-Fluorouracil sensitizes colorectal tumor cells towards double stranded
160 DNA breaks by interfering with homologous recombination repair. *Oncotarget* 6 (14), 12574-86.
- 161 4. Kufe, D.W. and Major, P.P. (1981) 5-Fluorouracil incorporation into human breast carcinoma RNA
162 correlates with cytotoxicity. *J Biol Chem* 256 (19), 9802-5.
- 163 5. Glazer, R.I. and Lloyd, L.S. (1982) Association of cell lethality with incorporation of 5-fluorouracil
164 and 5-fluorouridine into nuclear RNA in human colon carcinoma cells in culture. *Mol Pharmacol* 21
165 (2), 468-73.
- 166 6. Carter, J.M. et al. (2019) FICC-Seq: a method for enzyme-specified profiling of methyl-5-uridine in
167 cellular RNA. *Nucleic Acids Res.*
- 168 7. Kealey, J.T. et al. (1994) Enzymatic mechanism of tRNA (m5U54)methyltransferase. *Biochimie* 76
169 (12), 1133-42.

170 8. Hopper, A.K. et al. (1982) Defects in modification of cytoplasmic and mitochondrial transfer RNAs
171 are caused by single nuclear mutations. *Cell* 28 (3), 543-50.
172 9. Persson, B.C. et al. (1992) The gene for a tRNA modifying enzyme, m5U54-methyltransferase, is
173 essential for viability in *Escherichia coli*. *Proc Natl Acad Sci U S A* 89 (9), 3995-8.
174 10. Davanloo, P. et al. (1979) Role of ribothymidine in the thermal stability of transfer RNA as
175 monitored by proton magnetic resonance. *Nucleic Acids Res* 6 (4), 1571-81.
176 11. Asefa, B. et al. (1998) Genetic analysis of the yeast NUD1 endo-exonuclease: a role in the repair
177 of DNA double-strand breaks. *Curr Genet* 34 (5), 360-7.
178 12. Semionov, A. et al. (1999) Transient expression of *Saccharomyces cerevisiae* endo-exonuclease
179 NUD1 gene increases the frequency of extrachromosomal homologous recombination in mouse Ltk-
180 fibroblasts. *Mutat Res* 435 (2), 129-39.
181 13. Choudhury, S.A. et al. (2007) Functional and genetic analysis of the *Saccharomyces cerevisiae*
182 RNC1/TRM2: evidences for its involvement in DNA double-strand break repair. *Mol Cell Biochem* 300
183 (1-2), 215-26.
184 14. Guarguaglini, G. et al. (1997) Expression of the murine RanBP1 and Htf9-c genes is regulated
185 from a shared bidirectional promoter during cell cycle progression. *Biochem J* 325 (Pt 1), 277-86.
186 15. Chang, Y.H. et al. (2019) TRMT2A is a novel cell cycle regulator that suppresses cell proliferation.
187 *Biochem Biophys Res Commun* 508 (2), 410-415.

188