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COMMENTARY

An Alternative Molecular Mechanism of Action of 5-Fluorouracil, a Potent Anticancer Drug

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ABSTRACT. It is assumed that the primary mode of action of 5-fluorouracil (5-FUra) is mediated via inhibition of thymidylate synthetase. Persistent inhibition of cellular proliferation after treatment of the 5-FUra-inhibited cells with exogenous thymidine do not support the notion that the anti-proliferative action of 5-FUra is due exclusively to inhibition of DNA replication. Our studies have revealed an alternative mechanism of action at the level of pre-ribosomal RNA (pre-rRNA) processing. Pre-rRNA processing was inhibited completely *in vivo* as well as in S-100 extract from the mouse lymphosarcoma P1798 cells that were treated with 5-FUra. Under this condition, the 5-FUra-substituted pre-rRNA substrate was processed efficiently at the primary processing site. This study showed that the activity and/or the synthesis of a factor potentially involved in pre-rRNA processing is blocked in cells treated with the fluoropyrimidine. UV-cross-linking study showed that a 200 kDa polypeptide designated ribosomal RNA binding protein (RRBP) was absent in the S-100 extract from the drug-treated mouse lymphosarcoma cells. Since a polypeptide that cross-links to a processing site on RNA is usually involved in the RNA processing, RRBP may have a direct role in pre-rRNA processing. A key molecular mechanism for the antiproliferative action of 5-FUra may be due to its interference with the activity and/or synthesis of RRBP. Exposure of cells to 5-FUra did not inhibit the interaction between U3 small nucleolar RNA (snoRNA) and pre-rRNA at the primary processing site (a key step in the processing reaction) and the formation of U3 small nucleolar ribonucleoprotein (snoRNP). Treatment of cells with the fluoropyrimidine did not block the 3′ end processing of pre-messenger RNA (pre-mRNA). This article also discusses the effects of 5-FUra on pre-mRNA splicing and mRNA translation, and proposes other avenues of research to explore further the mechanism of action of this important pyrimidine analog. BIOCHEM PHARMACOL 53;11:1569–1575, 1997.

KEY WORDS. 5-fluorouracil; pre-rRNA processing; lymphosarcoma; splicing; U3 snoRNA; ribosomal RNA binding protein

5-FUra†, a pyrimidine analogue, is an important anticancer drug used widely in the treatment of a variety of solid tumors such as colorectal, breast, and liver carcinomas [1–4]. Although this potent drug has been used in cancer chemotherapy for nearly four decades, it major mechanism of action is still being debated. Elucidation of the primary mode of action of this drug is crucial for further development of more potent drugs with significantly reduced adverse reactions that are characteristic of most antineoplastic agents. It is assumed that the cytotoxic action of the fluorinated pyrimidine is mediated primarily via inhibition of thymidylate synthetase [5], the key enzyme that promotes the *de novo* synthesis of thymidylic acid which leads to the formation of dTTP, a substrate of DNA polymerase. Following transport into the cell, a significant portion of 5-FUra is converted to FdUMP by sequential action of pyrimidine phosphoribosyl transferase, nucleoside monophosphate kinase, and ribonucleotide reductase. FdUMP forms a ternary complex with the enzyme thymidylate synthetase and its cofactor 5,10-methylene tetrahydrofolate (see Fig. 1). This complex cannot break down further into separate entities, and as a result the enzyme is rendered inactive. The *de novo* synthesis of dTTP is blocked, which results in inhibition of DNA synthesis, a process called "thymineless death." Thymidylate synthase is overproduced in many 5-FUra-resistant cell lines, implying that this enzyme is indeed the target of its metabolite, FdUMP [6].

FdUMP is converted to FdUTP by the enzyme nucleoside monophosphate kinase. Recent studies have shown that FdUTP can be used as a substrate by mammalian DNA

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† *Abbreviations:* 5-FUra, 5-fluorouracil; rRNA, ribosomal RNA: rDNA, ribosomal RNA gene; pol I, RNA polymerase I; ETS1, 5′ external transcribed spacer; ETS2, 3′ external transcribed spacer; ITS1, internal transcribed spacer 1; ITS2, internal transcribed spacer 2; FUrd, 5 fluorouridine; pre-rRNA, pre-ribosomal RNA; snoRNA, small nucleolar RNA; RRBP, ribosomal RNA binding protein; snRNA, small nuclear RNA; snoRNP, small nucleolar ribonucleoprotein; pre-mRNA, pre-messenger RNA; DHFR, dihydrofolate reductase; FdUMP, 5-fluoro-2′-deoxyuridine 5′-monophosphate; FdUDP, 5-fluoro-2′-deoxyuridine 5′-diphosphate; FdUTP, 5-fluoro-2′-deoxyuridine 5′-triphosphate; FUMP, 5-fluorouridine 5′-monophosphate; FUDP, 5-fluorouridine 5′-diphosphate; and FUTP, 5-fluorouridine 5′-triphosphate.

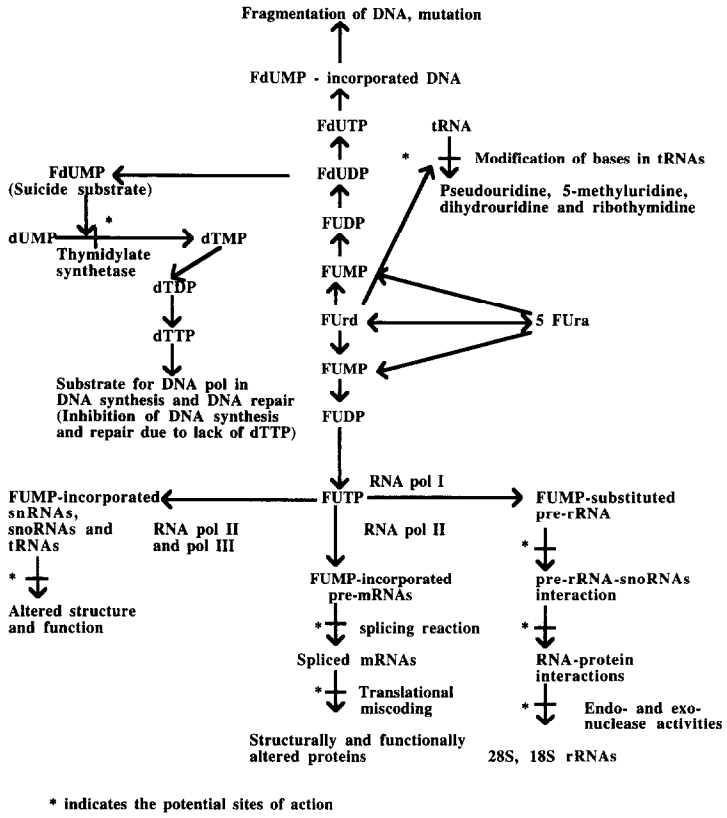

FIG. 1. Probable biochemical actions of 5-fluorouracil.

polymerases, causing misincorporation of FdUMP into DNA [4]. Although the misincorporated nucleotide can be removed by the cellular DNA repair system, 5-FUra-substituted DNA does not appear to be an ideal substrate for uracil-DNA glycosidase [7]. Incorporation of 5-FUra into DNA produces many deleterious effects, such as inhibition of further DNA synthesis [8], DNA fragmentations due to single- and double-strand breaks [9], and mutations induced by 5-FUra base pairing with guanine instead of adenine [10]. 5-FUra exposure also suppresses the DNA repair process due to dTTP depletion, which leads to generation of fragmented DNAs [9]. It is apparent from these studies that 5-FUra metabolites not only inhibit DNA synthesis but also cause other undesirable effects due to misincorporation of FdUTP into DNA.

Recent studies have cast doubt on the reduced synthesis of thymidylic acid as the only mechanism for the 5-FUra-induced inhibition of cellular proliferation. Significant suppression of cellular proliferation persisted after treatment of the 5-FUra-inhibited cells with exogenous thymidine or after simultaneous treatment with thymidine and 5-FUra [11]. In fact, addition of thymidine at certain concentrations increases antitumor activity of the drug in animal tumor models [12]. When the cells were treated first with methotrexate, an inhibitor of thymidylate synthetase, followed by 5-FUra treatment, the cell killing was synergistic, which is probably due to 5-FUra-mediated effects on RNA synthesis and/or processing [13]. The effect of 5-FUra on the erythroleukemic cell line K562 is solely mediated by interfering with rRNA processing rather than pre-rRNA synthesis [11]. Therefore, it should not be surprising that other mechanisms of action have been proposed for the inhibition of cellular proliferation and cytotoxicity of 5-FUra. These include alterations in mRNA expression [13–16], inhibition of mRNA splicing [17–20], and interference with tRNA modifications [21].

INHIBITION OF rRNA PROCESSING BY 5-FUra

Recent studies in our laboratory have revealed an interesting and novel mechanism of action of 5-FUra at the level of pre-rRNA processing. rDNAs of most eukaryotic cells are highly reiterated (50 to 2 × 10^6 gene copies/cell) and arranged in tandem array as head-to-tail repeats. They are transcribed by pol I in the nucleolus as a large (40–47S) precursor RNA (for reviews, see Refs. 22–24). The primary transcript starts with an ETS1 region, followed by 18S rRNA, an internal transcribed spacer (ITS1), the 5.8S RNA, another internal transcribed spacer (ITS2), the 28S RNA region, and finally an ETS2 region [25]. The transcript is subsequently processed into mature 28S, 18S, and 5.8S RNAs. 5S RNA, the remaining component of rRNA, is transcribed in the extra nucleolar compartment of the nucleus by RNA polymerase III. The primary processing of pre-rRNA occurs near the 5′ end of the ETS1 at a pair of sites approximately 5 nucleotides apart, which are located about 4 kb downstream of the transcription start site (see Fig. 2). The 5′ end cleaved product is then degraded rapidly, and the 3′ end product is processed further by endonucleolytic cleavages to produce the mature rRNAs.

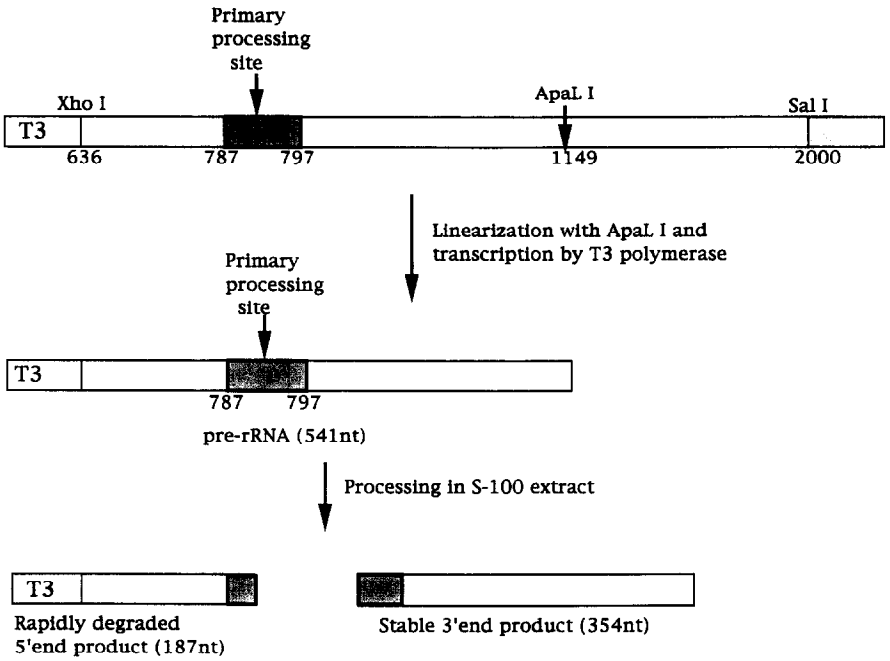

FIG. 2. Transcription of rat rRNA gene and its processing at the primary site. Precursor rRNA was synthesized by T3 polymerase from the cloned DNA containing the pre-rRNA processing site, and linearized with ApaL I (see Ref. 37 for details). Pre-rRNA was cleaved at the primary processing site (787–797 nucleotide region with respect to the transcription start site) in the S-100 extract prepared from the mouse lymphosarcoma P1798 cells. Two products, a rapidly degraded 187nt long 5′ end product and a stable 354nt 3′ end product, were generated. Treatment of these cells with 5-FUra resulted in the inhibition of pre-rRNA processing to form the stable 3′ end product.

Inhibition of pre-rRNA processing will affect adversely production of ribosomes, leading ultimately to arrest of protein synthesis. As early as 1973, 5-FUra was shown to inhibit pre-rRNA processing [26]. This study showed that exposure of cells to 5-FUra inhibited formation of mature 28S and 18S rRNAs, whereas synthesis of the 45S rRNA precursor proceeded unabated. In some tumor cell lines, the replacement of 5-FUra with its riboside analog, FUrd, augmented the inhibitory effect on rRNA synthesis [26]. Early electron microscope studies demonstrated 5-FUra-induced changes in the nucleolar size [27]. Similar observations were also made by using antibodies specific for nucleolar structures [28]. These may be related to reduction in pre-rRNA processing and the resultant accumulation of rRNA precursors in the nucleolus. Since 5-FUra can be readily incorporated into RNAs at a rapid rate to produce structurally modified molecules [21, 29–34], it is reasonable to speculate that 5-FUra-substituted rRNA may not be processed efficiently. Indeed, there is at least one report [35] that the inhibition of rRNA maturation by the fluorinated pyrimidine is contingent upon its incorporation into the rRNA precursor. On the other hand, Kanamaru *et al.* [36] followed the incorporation of [^{3}H]-5-FUra into pre-rRNA with time and showed that the pre-rRNA processing was blocked long after the radioactivity disappeared from the precursor RNA. This experiment indicated that the inhibition of pre-rRNA processing by 5-FUra is not dependent upon its prior incorporation into the RNA molecule mechanism by which the pyrimidine analogue inhibits cellular proliferation and exerts its cytotoxic effects has not been fully elucidated. Although 5-FUra can inhibit DNA replication as a result of the competitive inhibition of thymidylate synthetase, an alternate mechanism(s) of action appears to contribute significantly, if not primarily, to inhibition of cancer cell growth.

The discrepancy in the results from different laboratories is probably due to the use of less sophisticated techniques for exploring the effect of 5-FUra on the rRNA processing reaction. A definitive study requires a cell-free system that can be used to examine the effect of the anticancer drug on various trans-acting factors involved in the pre-rRNA processing reaction. Recently, our laboratory has developed an *in vitro* system from mouse lymphosarcoma P1798 cells that can efficiently process rat pre-rRNA [37]. Using this system, we demonstrated that transcription of rDNA or its subsequent processing at the primary processing site located between +787 and +797 was not inhibited when UTP was replaced with 5-FUTP in preparing the pre-rRNA substrate for the transcription reaction. This observation proves that incorporation of 5-FUMP into pre-rRNA does not inhibit its processing. The processing reaction, however, was inhibited completely when the cells were pretreated with 5-FUra before the extracts were made and used in the processing of exogenous pre-rRNA. Transcription of rRNA gene or the 3′ end processing of pre-mRNA (polyadenylation) proceeded unabated in these extracts [37]. These data imply that a factor(s) responsible for rRNA processing at the primary processing site is altered following treatment of the cells with 5-FUra and that this effect is specific to rRNA processing. Labeling of these cells with [^{32}P]orthophosphate showed that the synthesis of rRNA precursor (47S) remained unaffected after 5-FUra treatment, but its processing to 28S and 18S rRNAs was severely impaired (Ghoshal K, unpublished data).

A potential factor that may be affected by 5-FUra is U3 snoRNA that is known to be involved in pre-rRNA processing [38, 39]. U3 RNA is a 217-nucleotide long RNA associated with at least six proteins as a U3 snoRNP particle. The most well-characterized among the proteins is fibrillarin which is present as 2–10 $\times$ 10^{11} copies per

mammalian cell (for review, see Ref. 40). The role of U3 snoRNA in rRNA processing was demonstrated directly by inhibiting the processing reaction after immunodepletion of U3 snoRNP or after removal of U3 snoRNA by oligonucleotide-directed RNase H action [38]. U3 snoRNA has been shown to cross-link *in vivo* and *in vitro* to pre-rRNA sequence that spans the primary processing site [41–43]. The 5-FUra-induced reduction in pre-rRNA processing may be due to incorporation of the modified base into U3 RNA, which may prevent its association with the key polypeptides involved in the processing reaction or its interaction with the 5′ ETS region of pre-rRNA. The interaction of U3 snoRNA with pre-rRNA spanning the primary processing site was studied by *in vivo* psoralen cross-linking followed by northern blot analysis with antisense U3 snoRNA as probe. Psoralen has been shown to facilitate cross-linking between nucleic acids [44]. The cross-linking profile of U3 snoRNA from the cells treated with 5-FUra was very similar to that of the control cells (Ghoshal K, unpublished data). Cesium sulfate density gradient fractionation of the extracts prepared from the control and 5-FUra-treated cells followed by hybridization to antisense U3 snoRNA demonstrated no significant difference in the formation of U3 snoRNP particle in the two samples (Ghoshal K, unpublished data). This observation indicated that the association of U3 snoRNA with specific proteins to form the U3 snoRNP particles is not altered by 5-FUra treatment for 24 hr.

rRNA processing takes place within the "processosome" complex where many snoRNPs as well as some non-snoRNP proteins are involved in the cleavage of pre-rRNA at specific sites [24, 45]. Six polypeptides are known to cross-link specifically to mouse pre-rRNA spanning the primary processing site [46]. Among these polypeptides, two have been identified as nucleolin (110 kDa) and nucleolar endoribonuclease (52 kDa) [24, 46]. The role of nucleolin in rRNA processing is not known with certainty. The endoribonuclease, however, can catalyze processing of pre-rRNA at the primary site in the absence of any other factors *in vitro* [24]. We investigated whether 5-FUra treatment alters the activity and/or synthesis of these RNA binding proteins. For this purpose, the *in vitro* synthesized ^{32}P-labeled pre-rRNA fragment spanning the primary processing site was incubated with identical amounts of S-100 extracts prepared from the control or drug-treated cells under the optimal processing conditions [46]. The cross-linked proteins were separated by electrophoresis under denaturing conditions and visualized by autoradiography. Several proteins cross-linked to pre-rRNA fragment spanning the primary processing site, which could be competed out by unlabeled pre-rRNA, but not by nonspecific competitors such as poly(ACI) or tRNA. Among these proteins, a polypeptide with an approximate molecular mass of 200 kDa was either missing or severely reduced after the drug treatment (Ghoshal K, unpublished data). This data suggest that the 200 kDa protein designated RRBP is involved in pre-rRNA processing and that a probable mechanism of 5-FUra-mediated inhibition of the processing reaction is due to the decreased activity/amount of this protein. This experiment indicated the involvement of a specific protein in pre-rRNA processing and the effect of a potent anticancer drug on its activity and/or synthesis. The characteristics of this protein, its structure, and its role in pre-rRNA processing and in the 5-FUra-mediated inhibition of the reaction remain to be elucidated. The anticancer drug may inactivate this factor(s) or inhibit synthesis of the factor(s) via inhibition of transcription, splicing, translation of the mRNA, or post-translational modifications of the protein for the specific factor.

Treatment of mouse lymphosarcoma cells with 5-FUra did not inhibit the 3′ end processing of adenovirus L3 pre-mRNA. Neither the cleavage of the pre-mRNA at the polyadenylation site nor subsequent addition of poly(A) tail to the cleaved site was affected by the drug treatment. Those data demonstrated the specificity of the 5-FUra effect on pre-rRNA processing [37]. It is not known whether 5-FUra inhibits 3′ processing of other mRNA species.

EFFECT OF 5-FUra ON pre-mRNA SYNTHESIS, SPLICING, AND TRANSLATION

Unlike the *Drosophila* system [47], mammalian RNA polymerase can efficiently use 5-FUTP as the substrate. Different investigators claimed its stimulatory or inhibitory effect on different RNA polymerase II-directed genes, based on the steady-state levels of different mRNA transcripts by northern blot analysis. Dolnick and Pink [48] observed significant accumulation of DHFR mRNA in the methotrexate-resistant human cell line KB7B after treatment with 5-FUra in the presence of excess thymidine. Similarly, Heimer and Sartorelli [11] investigated the effect of FUrd in the presence of excess thymidine on the expression level of different protein coding genes in K562 cells where only minimal inhibition of thymidylate synthetase was observed. Under these conditions, cellular mRNA levels for c-myc, c-abl and β-globin decreased significantly, whereas the levels of actin remained unaltered and that of ornithine decarboxylase increased. These studies do not pinpoint the differential effect of FUrd treatment on the transcription of different protein-coding genes. Alternatively, the accumulation or disappearance of different mRNAs may also be due to alteration in the post-transcriptional processing of the pre-mRNAs for these genes following treatment with 5-FUra [13–16]. Growth of cells for a long period at low levels of the fluoropyrimidine led to accumulation of DHFR pre-mRNAs containing contiguous exons and introns, which clearly suggested inhibition of pre-mRNA splicing in the drug-treated cells [14, 48]. The splicing reaction could be inhibited by two possible mechanisms. Incorporation of 5-FUra into the body of pre-mRNA could alter its structure so that it is not recognized and processed by the splicing machinery. Alternatively, incorporation of the drug into a component(s) of the splicing machinery could render it

inactive, leading to inhibition of splicing. The data gathered to date do not seem to support the first hypothesis. When 5-FUra-substituted β-globin pre-mRNA fragment generated by *in vitro* transcription with phage RNA polymerase was incubated with HeLa nuclear extract, the majority of the spliced products was correct [18], although a minor aberrant spliced product was generated under certain conditions [17]. 5-FUra treatment inactivated the splicing efficiency of HeLa nuclear extract in a time- and dose-dependent manner [18]. A strong candidate for the target of 5-FUra in the splicing reaction is the large RNA–protein complex called "Splicesome," which consists of small nuclear U-rich RNA containing RNP particles designated "snurp" and many non-snurp proteins in addition to pre-mRNA. snRNAs play a very important role not only in splicesome formation but also in the catalytic reactions such as excising the introns and joining the exons [49]. It is logical to conceive that the incorporation of 5-FUra into these U-rich snRNAs may alter their structure, which could adversely affect the splicing machinery. Indeed, a significant reduction in the level of U2 snRNA has been reported [18], which may explain the 5-FUra-induced inhibition of the splicing reaction. On the other hand, Patton [20] did not observe a change in the level of snRNAs or their ability to form snurp particles after the drug treatment. Studies in the yeast splicing system provided the conclusive evidence for alteration in the structure and function of U2 snurp following incorporation of 5-FUra into the snRNA moiety. Depletion of specific snRNAs from the yeast whole cell extract proficient in splicing by specific oligodeoxynucleotide-directed RNase H digestion inactivated the splicing activity, which could be restored by the addition of *in vitro* synthesized snRNA. Addition of 5-FUra-substituted U6 snRNA to the extract depleted of U2 and U6 snRNAs separately resulted in significant restoration of the splicing activity, whereas inhibition of the splicing activity persisted upon addition of 5-FUra-substituted U2 snRNA [19]. This study also showed that incorporation of the fluorinated pyrimidine into U2 snRNA altered its secondary or tertiary structure, which appears to be responsible for its inability to form specific snurp particles and spliceosomal complexes. It forms abnormal complexes with pre-mRNA and other proteins, which results in rapid degradation of pre-mRNA [19]. It is not known whether the incorporation of 5-FUra into snRNAs other than the U2 and U6 species leads to deleterious effects on pre-mRNA splicing.

The incorporation of 5-FUra into mRNA could induce translational miscoding, as 5-FUra may base pair with guanine instead of adenine. Dihydrofolate reductase mRNA isolated from KB cells exposed to 5-FUra was translated *in vitro* at a rate equal to that of mRNA from the control cells [13]. However, thymidylate synthetase from the drug-treated extract could not be inhibited significantly either by methotrexate or by a monospecific antiserum against the wild-type enzyme, although the size of the protein remained unaltered. This study suggests that replacement of UMP with 5-FUMP in mRNAs could produce altered proteins. Alteration in protein synthesis could also occur as a result of inhibition of uridine modifications of tRNAs by 5-FUra [21, 34].

CONCLUSIONS AND PERSPECTIVES

Although competitive inhibition of thymidylate synthetase by 5-FUra is a major mode of action of this fluoropyrimidine, this effect alone cannot explain its cytotoxicity or the antitumor action. Since exogenous thymidine does not prevent the inhibitory effects of 5-FUra on cell proliferation and, in fact, enhances the cytotoxicity of the drug, an alternative mechanism of action for this potent compound has been explored in many laboratories. The present study has demonstrated that treatment of cells with 5-FUra results in inhibition of pre-rRNA processing. The 5-FUra-induced arrest of pre-rRNA processing could inhibit production of functional ribosomes that are essential for protein synthesis. Neither pre-rRNA synthesis nor the 3′ end processing of pre-mRNA is suppressed under this condition. Specific inhibition of pre-rRNA processing by the fluoropyrimidine may not be the sole factor responsible for the anticancer action of 5-FUra, and the drug-induced cell death may be mediated by a combination of events. If the protein RRBP is indeed a key factor involved in the processing of pre-rRNA at the primary 5′ site of pre-rRNA, its activity in the 5-FUra-resistant cell lines should be explored. If pre-rRNA processing continues unabated in any of the resistant cell lines, the role of pre-rRNA processing in the 5-FUra-mediated cytotoxic effects and inhibition of cellular proliferation could emerge as a key molecular mechanism of action of the drug. Although short-term treatment of cells (24 hr) with 5-FUra did not affect the interaction between U3 snoRNA and pre-rRNA region spanning the primary processing site, the drug may inhibit the RNA–RNA interaction upon prolonged treatment. Numerous snoRNAs are being discovered, and the role of some of these U-rich RNAs (U8, U14, U22, snR30) in rRNA processing has been established [24, 45]. It would be of interest to examine the effect of 5-FUra treatment on the different steps in pre-rRNA processing mediated by these snoRNAs.

Finally, the antiproliferative action of 5-FUra may be due to activation of tumor suppressor genes or inactivation of specific oncogenes. 5-FUra treatment may also lead to apoptosis or programmed cell death. Indeed, treatment of the rat prostatic cells (AT-3) with FUrd can cause significant overexpression of the prostate-specific protein TRPM-2 (testosterone-repressed prostatic message-2) [50]. Although the exact function of this protein is unknown, its induction results in apoptosis of the cancer cells. Therefore, it is conceivable that 5-FUra or its metabolites may affect the expression of specific proteins. Further studies are likely to address these important issues concerning the action of 5-FUra.

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References

1. Tobias JS, Current role of chemotherapy in head and neck cancer. *Drugs* **43:** 333–345, 1992.
2. Mayer RJ, Systemic therapy for colorectal cancer: An overview. *Semin Oncol* **18:** 62–66, 1991.
3. Grem JL, Hoth DF, Hamilton JM, King SA and Leyland-Jones B, Overview of current status and future direction of clinical trials with 5-fluorouracil in combination with folinic acid. *Cancer Treat Rep* **71:** 1249–1264, 1987.
4. Weckbecker G, Biochemical pharmacology and analysis of fluoropyrimidines alone and in combination with modulators. *Pharmacol Ther* **50:** 367–424, 1991.
5. Santi DV, McHenry CS and Sommer H, Mechanism of interaciton of thymidylate synthetase with 5-fluorodeoxyuridylate. *Biochemistry* **3:** 471–481, 1974.
6. Zhang Z-G, Harstrick A and Rustum YM, Mechanisms of resistance to fluoropyrimidines. *Semin Oncol* **19:** 4–9, 1992.
7. Caradonna SJ, and Cheng Y-C, The role of deoxyuridine triphosphate nucleotidohydrolase, uracil-DNA glycosylase, and DNA polymerase α in the metabolism of FUdR in human tumor cells. *Mol Pharmacol* **18:** 513–520, 1980.
8. Schuetz JD, Wallace HJ and Diasio RB, 5-Fluorouracil incorporation into DNA of CF-1 mouse bone marrow cells as a possible mechanism of toxicity. *Cancer Res* **44:** 1358–1363, 1986.
9. Lonn U, Lonn S, Nylen U and Winblad G, 5-Fluoropyrimidine-induced DNA damage in human colon adenocarcinoma and its augmentation by the nucleoside transport inhibitor dipyridamole. *Cancer Res* **49:** 1085–1089, 1989.
10. Herrington MB and Takahashi I, Mutagenesis of bacteriophage PBS2. *Mutat Res* **20:** 275–278, 1973.
11. Heimer R and Sartorelli AC, RNA-directed actions of 5-fluorouridine in hemin stimulated K-562 erythroleukemia cells. *Cancer Biochem Biophys* **12:** 221–239, 1992.
12. Spiegelman S, Nayak R, Sawyer R, Stolfi R and Martin D, Potentiation of the anti-tumor activity of 5FU by thymidine and its correlation with the formation of (5FU)RNA. *Cancer* **45:** 1129–1134, 1980.
13. Dolnick BJ and Pink JJ, Effects of 5-fluorouracil on dihydrofolate reductase and dihydrofolate reductase mRNA from methotrexate-resistant KB cells. *J Biol Chem* **260:** 3006–3015, 1985.
14. Will CL and Dolnick BJ, 5-Fluorouracil augmentation of dihydrofolate reductase transcripts containing intervening sequences in methotrexate-resistant KB cells. *Mol Pharmacol* **29:** 643–648, 1986.
15. Will CL and Dolnick BJ, 5-Fluorouracil augmentation of dihydrofolate reductase RNA containing contiguous exon and intron sequences in KB7B cells. *J Biol Chem* **262:** 5433–5436, 1987.
16. Armstrong RD, Lewis M, Stern SG and Cadman EC, Acute effect of 5-fluorouracil on cytoplasmic and nuclear dihydrofolate reductase messenger RNA metabolism. *J Biol Chem* **261:** 7366–7371, 1986.
17. Doong SL and Dolnick BJ, 5-Fluorouracil substitution alters pre-mRNA splicing *in vitro*. *J Biol Chem* **263:** 4467–4473, 1988.
18. Sierakowska H, Shukla RR, Dominski Z and Kole R, Inhibition of pre-mRNA splicing by 5-fluoro-, 5-chloro-, and 5-bromouridine. *J Biol Chem* **266:** 19185–19191, 1989.
19. Lenz H, Manno DJ, Danenberg KD and Danenberg PV, Incorporation of 5-fluorouracil into U2 and U6 snRNA inhibits mRNA precursor splicing. *J Biol Chem* **269:** 31962–31968, 1994.
20. Patton JR, *In vitro* splicing of pre-messenger RNA with extracts from 5-fluorouridine-treated cells. *Biochem J* **297:** 297–301, 1994.
21. Tseng WC, Medina D and Randerath K, Specific inhibition of transfer RNA methylation and modification in tissues of mice treated with 5-fluorouracil. *Cancer Res* **38:** 1250–1257, 1978.
22. Larson DE, Zahradka P and Sells BH, Control points in eukaryotic ribosome biogenesis. *Cell Biol* **69:** 5–22, 1991.
23. Jacob ST, Regulation of ribosomal gene transcription. *Biochem J* **306:** 617–626, 1995.
24. Eichler DC and Craig N, Processing of eukaryotic ribosomal RNA. *Prog Nucleic Acid Res Mol Biol* **49:** 199–237, 1994.
25. David IB and Wellauer DK, A reinvestigation of 5′–3′ polarity in 40S ribosomal RNA precursor on *Xenopus laevis*. *Cell* **8:** 443–448, 1976.
26. Wilkinson DS and Pitot HC, Inhibition of ribosomal ribonucleic acid maturation in Novikoff hepatoma cells by 5-fluorouracil and 5-fluorouridine. *J Biol Chem* **248:** 63–68, 1973.
27. Stenram U, The effects of fluorouracil and actinomycin, single and combined, on the nucleolar ultrastructure of various tissues of the rat. *Z Zellforsch Mikrosk Anat* **94:** 282–292, 1969.
28. Greenhalgh DA and Parish JH, Effect of 5-fluorouracil combination therapy on RNA processing in human colonic carcinoma cells. *Br J Cancer* **61:** 415–419, 1990.
29. Carrico CK and Glazer RI, Augmentation of the incorporation and distribution of 5-fluorouracil in ribosomal RNA. *Biochem Biophys Res Commun* **87:** 664–760, 1979.
30. Glazer RI and Hartman KD, Analysis of the effect of 5-fluorouracil on the synthesis and translation of polysomal poly(A)RNA for Ehrlich ascites cells. *Mol Pharmacol* **19:** 117–121, 1981.
31. Glazer RI and Legraverend M, The effect of 5-fluorouridine 5′-triphosphate on RNA transcribed in isolated nuclei *in vitro*. *Mol Pharmacol* **17:** 279–282, 1980.
32. Glazer RI and Peale AL, The effect of 5-fluorouracil on the synthesis of nuclear RNA in L-1210 cells *in vitro*. *Mol Pharmacol* **16:** 270–277, 1979.
33. Heidelberger C, Fluorinated pyrmidines and their nucleosides. In: *Antineoplastic and Immunosuppressive Agents* (Eds. Sartorelli AC and Johns DG) Vol. 2, pp. 193–231. Springer, New York, 1975.
34. Lu LW, Chiang GH, Tseng WC and Randerath K, Effects of 5-fluorouridine on modified nucleotides in mouse liver transfer RNA. *Biochem Biophys Res Commun* **73:** 1075–1082, 1976.
35. Herrick D and Kufe DW, Lethality associated with incorporation of 5-fluorouracil into preribosomal RNA. *Mol Pharmacol* **26:** 135–140, 1984.
36. Kanamaru R, Kakuta H, Sato T, Ishioka C and Wakui A, The inhibitory effects of 5-fluorouracil on the metabolism of pre-ribosomal and ribosomal RNA in L-1210 cells *in vitro*. *Cancer Chemother Pharmacol* **17:** 43–46, 1986.
37. Ghoshal K and Jacob ST, Specific inhibition of pre-ribosomal RNA processing in extracts from the lymphosarcoma cells treated with 5-fluorouracil. *Cancer Res* **54:** 632–636, 1994.
38. Kass S, Tyc K, Steitz JA and Sollner-Webb B, The U3 small nuclear ribonucleoprotein function in the first step of preribosomal RNA processing. *Cell* **60:** 897–908, 1990.
39. Beltrame M and Tollervey D, Identification and function analysis of two U3 binding sites on yeast pre-ribosomal RNA. *EMBO J* **11:** 1531–1542, 1992.
40. Reddy R and Busch H, Small nuclear RNAs: RNA sequences, structure and modifications. In: *Structure and Functions of*

Major and Minor Small Nuclear Ribonucleoprotein Particles (Ed. Birnsteil ML), pp. 1–37. Springer, Berlin, 1988.
41. Stroke IL and Weiner AM, The 5′ end of U3 snRNA can be crosslinked *in vivo* to the external transcribed spacer of rat ribosomal RNA precursors. *J Mol Biol* **210:** 497–512, 1989.
42. Maser RL and Calvet JP, U3 small nuclear RNA can be psoralen-cross-linked *in vivo* to the 5′ external transcribed spacer of pre-ribosomal RNA. *Proc Natl Acad Sci USA* **86:** 6523–6527, 1989.
43. Tyc K and Steitz JA, A new interaction between the mouse 5′ external transcribed spacer of pre-rRNA and U3 snRNA detected by psoralen crosslinking. *Nucleic Acids Res* **20:** 5375–5382, 1992.
44. Calvet JP and Pederson T, Base-pairing interactions between small nuclear RNAs and nuclear RNA precursors as revealed by psoralen cross-linking *in vivo*. *Cell* **26:** 363–370, 1981.
45. Lafontaine D and Tollervey D, Transacting factors in yeast pre-rRNA and pre-snoRNA processing. *Biochem Cell Biol* **73:** 802–812, 1996.
46. Kass S and Sollner-Webb B, The first processing event occurs in a large complex: Analysis by gel retardation, sedimentation and UV crosslinking. *Mol Cell Biol* **10:** 4920–4931, 1990.
47. Lengyel J and Penman S, hnRNA size and processing as related to different DNA content in two Dipterans: Drosophila and Aedes. *Cell* **5:** 281–290, 1975.
48. Dolnick BJ and Pink JJ, 5-Fluorouracil modulation of dihydrofolate reductase RNA levels in methotrexate-resistant KB cells. *J Biol Chem* **258:** 13299–13306, 1983.
49. Guthrie C, Messenger RNA splicing in yeast: Clues to why the spliceosome is a ribonucleoprotein. *Science* **253:** 157–163, 1991.
50. Kyprianou N and Isaacs JT, "Thymineless" death in androgen-independent prostatic cancer cells. *Biochem Biophys Res Commun* **165:** 73–81, 1989.