Chapter 6. The 5-fluorouracil Story 220927dh3

Drugs Against Cancer: Stories of Discovery and the Quest for a Cure

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CHAPTER 6

The 5-Fluorouracil Story: from a simple idea to a major anti-cancer drug.[1]

The molecule was simple, as was the idea it was based on, but its impact on cancer therapy was profound. The idea was merely to add a small fluorine atom to uracil, one of the building blocks for the production of RNA and DNA (Figure 6.1). The product, 5-fluorouracil (5FU), despite the minimal change in chemical structure, turned out to disturb DNA and RNA production in a surprisingly complex manner. 5FU was found to be toxic to rapidly dividing cancer cells, and the effects of 5FU on the RNA and DNA synthesis pathways were worked out, but exactly how these actions produced the drug's anti-cancer activity remained obscure.

Discovery through knowledge and intuition

5-Fluorouracil (5FU) was one of the first anti-cancer drugs to be discovered (after nitrogen mustard and methotrexate) and one of the most important. The story began in 1954 at the McArdle Memorial Laboratory in Madison, Wisconsin, with the discovery by Charles (Charlie) Heidelberger that adding a fluorine atom to uracil yielded a compound that had anti-cancer activity in mice and rats (Heidelberger et al., 1957) (Figure 6.2). The fluorine atom was cleverly placed at the 5-position, which, as we shall see, turned out to be critical for its anti-cancer action. The intuition to place a fluorine atom on the 5-position, came from the idea that thymine, a critical constituent of DNA but not RNA, had a methyl group on that 5-position of uracil: the cell made thymine from uracil by adding a methyl group to uracil

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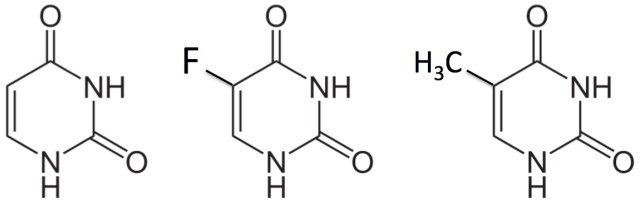
(actually, by adding a methyl to a nucleotide of uracil to make a nucleotide of thymine).

Charlie Heidelberger, son of the famous immunologist Michael Heidelberger, was a major figure in cancer research, noted for his sharp mind and demand for research excellence, until his untimely death at the age of 63, ironically of cancer. He was much loved and respected, but also feared by some younger researchers, because of his challenging questions and comments when in sessions that he chaired. (I was in fact one of his victims in 1961, when, during the discussion period after my talk, I claimed too much future progress, whereupon he said, "I congratulate Dr. Kohn ahead of time for his future success." It was a lesson I never forgot.)

Experience had shown that adding a fluorine atom, even though it is one of the smallest atoms, sometimes changed the properties of a drug. With that in mind, Heidelberger focused on uracil as a target compound, because of an idea that was circulating at the time that cancers might incorporate uracil into RNA more actively than did normal tissues. But, where on the uracil molecule would he add the fluorine? Insightfully, as already mentioned, he chose the 5-position, because that is where thymine had a methyl group (Figure 6.1), and he may have conjectured that the fluorine might then somehow interfere with the production or function of thymine, an essential component of DNA. He was absolutely right! But the way 5FU caused its effects turned out to be much more complicated than anticipated (Longley et al., 2003).

When clinical investigators at McArdle in 1958 in the first clinical trial (prior FDA approval was not yet required), administered 5FU to patients who had a variety of cancers, they found that some of the patients' tumors showed signs of regression, but this occurred only when the drug dose was high enough to produce severe toxicity (Curreri et al., 1958). (Note how quickly – within just a few years – discovery progressed to successful testing in patients.) In 1962, clinical researchers at the University of Wisconsin reviewed the clinical experience with 5FU up to that time. They noted that, although some of the published reports failed to find significant benefit, taking all the results together and focusing on patients whose tumors were large enough to be measured, about 21% of patients who received the drug to the point of mild toxicity had a reduction in the size of the tumor, and that the remission lasted an average of 9 months (Ansfield et al., 1962). At the time, that was a significant effect against hard-to-treat cancers. Extensive clinical studies were then carried out to determine the best dosage schedule (the timing and amount of successive doses) in various types of cancer, and it was found that the drug was especially effective against colon cancer (Ansfield, 1964) (Ansfield et al., 1977). We will see later in this chapter how new therapies based on 5FU had much better outcomes.



Uracil 5-Fluorouracil Thymine

Figure 6.1. Uracil is a building block for the production of RNA; in the cell, a methyl group (CH_3) is added at position 5 of uracil, producing thymine building blocks for DNA. 5-Fluorouracil (5FU) is simply uracil with a fluorine atom (F) added at the location (position 5) where thymine has a methyl group.

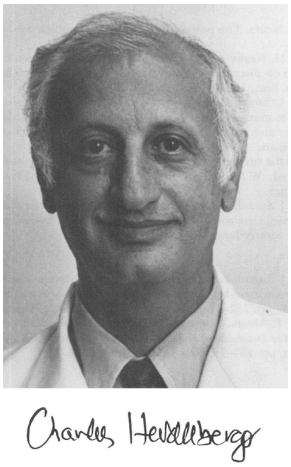


Figure 6.2. Charles ("Charlie") Heidelberger (1920-1983). *(Photo from a Biographical Memoir, National Academy of Sciences, Washington DC, 1989).)*

Improving the effectiveness of 5-fluorouracil (5FU) in cancer treatment.

Since the therapeutic ability of 5-FU by itself was meager (response rates of only 10 to 15% in colon cancer (Longley et al., 2003)), much effort was put into making the drug more effective. The first improvement was to use leucovorin (folinic acid) (see Chapter 5), the natural form of folic acid, together with 5FU. That combination enhanced 5FU's cell killing action. The cell converts leucovorin to methylene-tetrahydrofolate, which is required at high concentration to effect the binding of 5FU to thymidylate synthase (Longley et al., 2003). How leucovorin acts together with 5FU to inhibit thymidylate synthase will be explained later in this chapter.

The addition of leucovorin to 5FU doubled the response rate of colon cancer, compared to 5FU alone (from about 11% to about 23%), but it unfortunately had little effect on survival time (Longley et al., 2003). Killing most of the cancer cells

was able to improve quality of life temporarily, but the remaining malignant cells eventually grew into new tumors that no longer responded to the drugs.

In another report, adding leucovorin to 5FU significantly increased the survival of patients with advanced colon cancer with distant metastases (stage IV). However, the chance of surviving one year with either treatment was not very good, and the chance of surviving 2 years was dismal (Figure 6.3) (Poon et al., 1989).

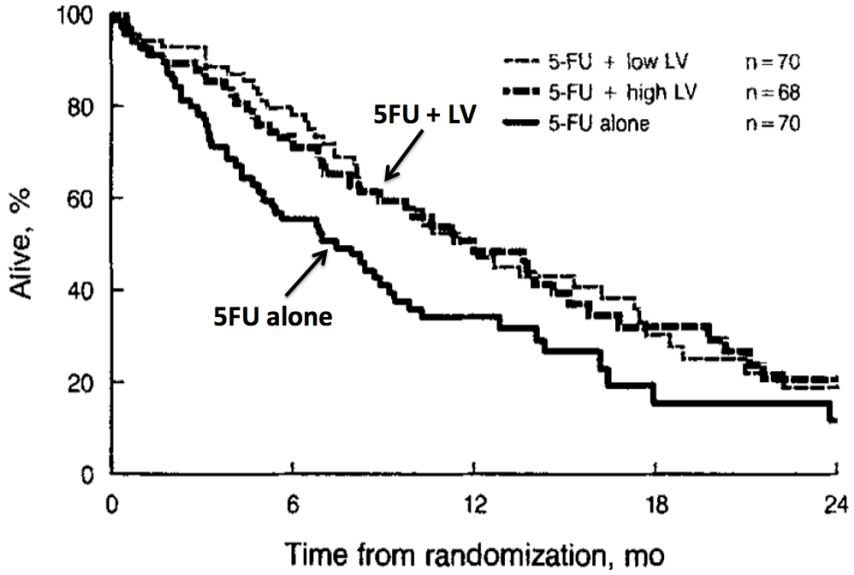


Figure 6.3. Survival of colorectal cancer patients who already had metastases (stage IV) or whose tumor could not be removed by surgery. The horizontal axis shows number of months after treatment began. The survival of patients treated with 5-fluosoruracil (5FU) alone is shown by the lower curve. The upper curves show the survival of patients who received leucovorin (2 dosage levels) in addition to 5FU (Poon et al., 1989). Very few patients survived for 24 months, regardless of which treatment was given.

After surgical removal of a colon cancer that had not metastasized to distant sites, patients often continued to be treated with drugs, usually including 5FU or one of its relatives ("adjuvant chemotherapy"). Patients who had extensive regional lymph node involvement, but no distant metastases (advanced stage III) had a much better outlook than those whose cancer had already metastasized to distant sites. Their long-term survival after surgery followed by 5FU plus leucovorin was about 50%, which was increased to about 60% if oxaliplatin also was added. Oxaliplatin (see Chapter 3) seemed to have been effective only on the more advanced parts of the cancer, because it did not benefit patients with earlier stages of the disease (Figure 6.4).

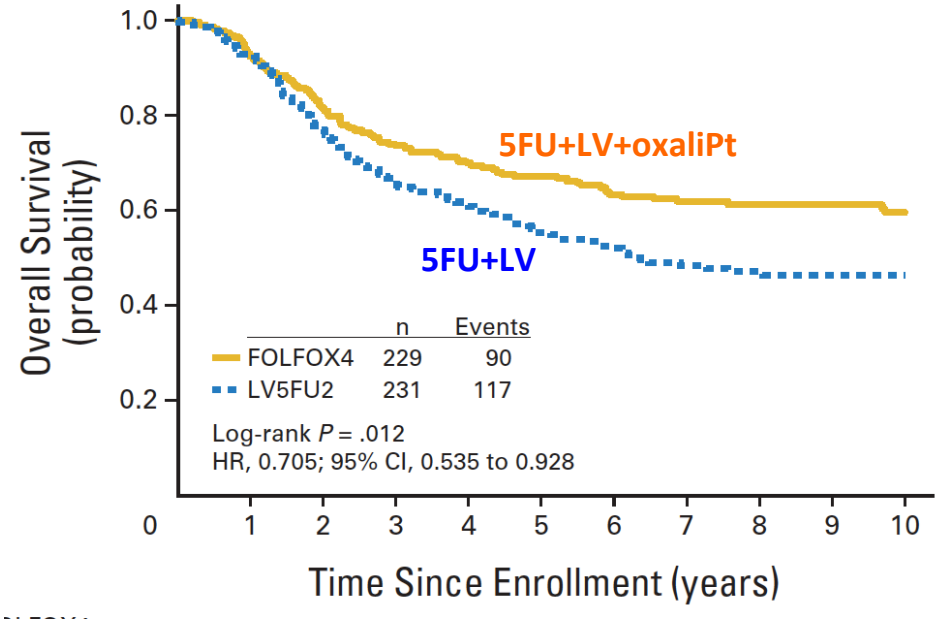


Figure 6.4. Long-term survival of advanced stage III colorectal cancer patients (tumor in many regional lymph nodes, but no distant metastases). Patients in this group who were treated with 5FU+leucovorin had about 50% chance of surviving 10 years; if oxaliplatin was added to the adjuvant therapy of 5FU+leucovorin, the survival probability rose to about 60%. Oxaliplatin however did not benefit patients with less advanced disease (Andre et al., 2015).

How could one tell who needed adjuvant therapy (continued chemotherapy after complete surgical removal to the tumor) and who did not? A blood test was developed that was promising. The test used a highly sensitive DNA analysis method to detect cancer cells or their DNA in the blood. If cancer DNA was detected in the blood of stage II colon cancer patients, it was surmised that they would benefit from adjuvant chemotherapy (Figure 6.5) (Tie et al., 2016). However, a small fraction of the patients who had no detectable cancer DNA in the blood, did have recurrence of the cancer, and they might also have been helped by adjuvant chemotherapy. This is a situation where patients would be called upon to make the decision, based on their consideration of risk and toxicity versus benefit.

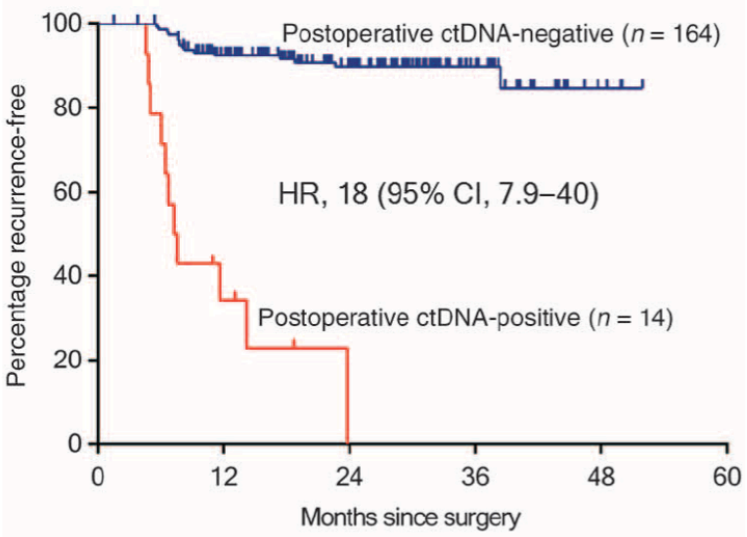


Figure 6.5. Patients with stage II colon cancer who did not receive adjuvant chemotherapy, and who had detectable cancer DNA circulating in the bloodstream, were at risk of soon having a recurrence of the cancer (lower curve, 14 patients). Similar patients who did not have detectable cancer DNA in the blood had a high likelihood of being cured (upper curve, 164 patients) (Tie et al., 2016). (Note that only 8% of patients had detectable cancer DNA in the blood, and these patients were at risk and would probably have benefited from adjuvant chemotherapy; most of those who did not have cancer DNA in the blood did not need adjuvant chemotherapy.) *(www.ScienceTranslationalMedicine.org 6 July 2016 Vol 8 Issue 346 346ra92).*

Capecitabine: a pro-drug for 5FU

A difficulty for therapy with 5FU was that it was rapidly destroyed by enzymes in the blood, and therefore had to be administered around the clock. Much of the intravenously administered 5FU was destroyed in the blood before the drug entered the cell. Capecitabine was developed as a pro-drug that is converted to 5FU by enzymes in the cell. It was not destroyed in the blood, entered cells and only then was converted to 5FU. Moreover, unlike 5FU, it could be given orally, a major practical advantage during treatment. The new drug was itself inactive (it was a "prodrug") and had to be activated by reactions, first in the liver and then by enzymes that are highly active in some cancers. Thus the active 5FU was generated right in the tumor cell (Miwa et al., 1998) (Johnston and Kaye, 2001).

Capecitabine was inactive, because of the side chain shown at the top of the structure in Figure 6.6. Enzymes in the liver and cancer cell removed the side chain to yield bare 5-fluorouracil, which formed directly in the cell, thereby evading destruction by enzymes outside of the cell.

Despite its theoretical advantages, however, capecitabine produced only a modestly higher response rate than 5FU in colon cancer with somewhat less toxicity. But it unfortunately had little effect on the survival time of patients (Longley et al., 2003). It nevertheless had the benefit of oral rather than prolonged intravenous administration.

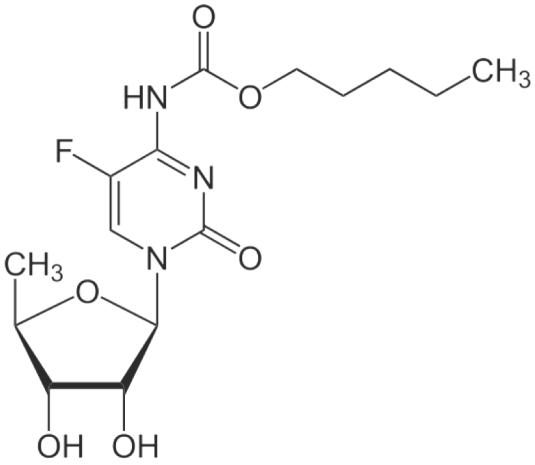


Figure 6.6. Structure of capecitabine, an inactive form of 5FU (a pro-drug), which is converted by 5FU by enzymes, first in the liver, and then in the cell. Active 5FU thus forms directly in the cell. The activation occurs by removal of the inactivating chain from the amino group (NH) at the top. Capecitabine had the advantage that it was not destroyed in the blood and oral administration was effective. But the hoped-for benefit in terms of prolonging the lives of cancer patients was disappointing.

How colon cancer came to be treated with 5-Fluorouracil (5FU).

We will look back now on the history of this dreadful disease and its treatment. Colorectal cancer, at least up to 2015, was the 4th most common cancer, after prostate cancer in men, breast cancer in women, and lung cancer in both sexes. Also, it was the 3rd most frequent cause of cancer-related death in the United States.

Figure 6.7 gives an idea of the culprit that had to be deal with; it shows a typical view (histology) of the cells in a moderately differentiated colon cancer, which was the most common type. Cancers that were poorly differentiated (few gland-like structures) were more aggressive and had a worse prognosis than tumors that were highly differentiated (many gland-like structures) (Fleming et al., 2012).

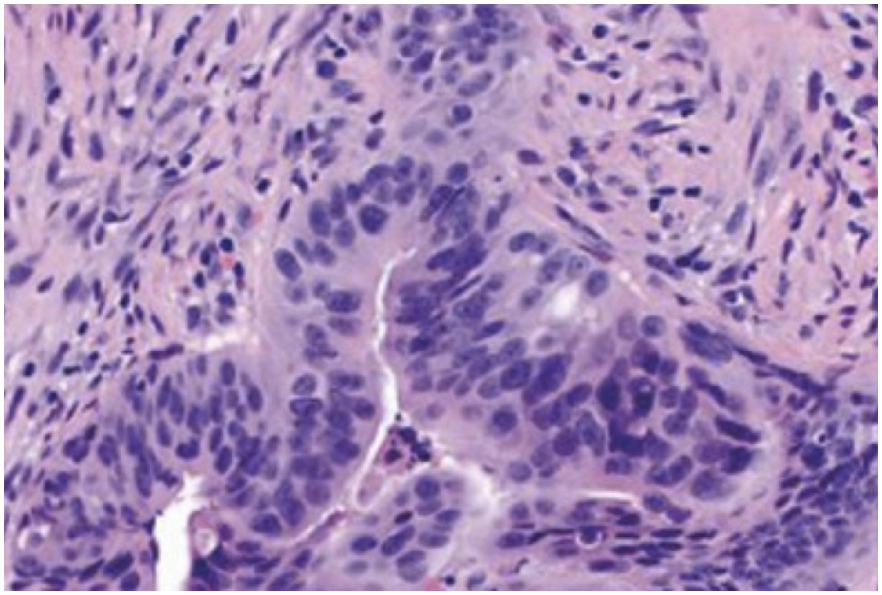


Figure 6.7. A typical picture of the culprit: a moderately differentiated colon carcinoma. Many of the cancer cells (cells with large nuclei) are arranged in a manner resembling the gland structure of the colon, but in a disorganized fashion: large-scale tissue order is lost. The tumors are surrounded by fibrous tissue cells (stroma), which may contribute to the malignancy of the cancer (Fleming et al., 2012). *(Fleming M, Ravula S, Tatishchev SF, Wang HL. Colorectal carcinoma: Pathologic aspects. Journal of Gastrointestinal Oncology. 2012;3(3):153-173).*

Colon cancers were noted to be of two general types, depending on where in the colon they arose. From the point where the small intestine joins the colon in the lower right side of the abdomen, the colon ascends on the right, crosses over the midline and descends on the left side to the rectum. The first ("proximal") and second ("distal") parts of the colon along that path have differences that trace back to the way they form in the embryo (Bufill, 1990). The two sections of the colon are like different tissues and have different types of cancers with different drug sensitivities.

Most colon cancers arise in pre-malignant outgrowths, called polyps; these are found only in the distal (descending on the left side) colon, and could be removed during colonoscopy. That was important, because it is within those polyps that the great majority (about 80%) of colon cancers developed (Figure 6.8). Fortunately, malignant cancers in those polyps take years to develop, which gave time for them to be removed during regular colonoscopy. However, a few people have a rare inherited mutation of the *APC* (adenopolyposis coli) gene that caused continuous formation of many polyps that had to be removed by frequent colonoscopies.

The cancers arising in polyps in the distal colon usually responded to 5FU. On the other hand, the less common cancers in the proximal (ascending on the right side) colon did not form polyps, and rarely responded to 5FU (Carethers et al., 2004; Kawakami et al., 2015). Another difference was that cancers in the proximal colon often had a mutation in one of the DNA mismatch repair (MMR) genes (discussed in Chapter 25).

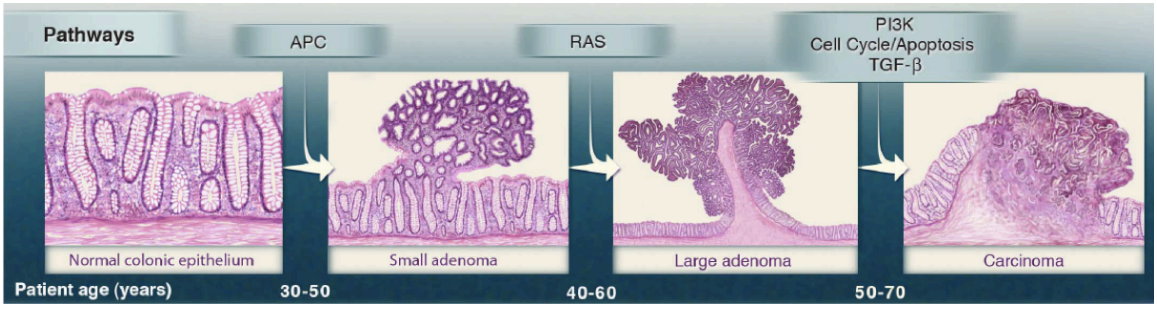


Figure 6.8. How cancer develops from a polyp in the distal (descending) colon, as conceptualized by Bert Vogelstein (Vogelstein et al., 2013). Shown here are the stages from normal to cancerous polyps as conceived by Vogelstein. In the normal colonic epithelium (left), a polyp develops with a small adenoma in it (second picture from the left); this happens occasionally when there is a rare mutation in the APC (adenopolyposis coli) gene. After several years, cells in this still benign but pre-cancerous adenoma may acquire a mutation in the RAS gene, which then allows the tumor to grow to become a large adenoma (third picture from the left). Up to this stage, the tumor is still benign and could be removed during colonoscopy. After several more years, the tumor may invade deeper tissues (right) and become malignant and could metastasize. As long as the cancer remained local and without spread to the regional lymph nodes, it was stage II and could be cured by surgery with or without adjuvant drug therapy (continued drug therapy after surgical removal of the tumor). If it had spread to the regional lymph nodes, but not yet metastasized to other organs, it was stage III and could still often be cured by surgery followed by adjuvant drug therapy. For cancers that had metastasized, for example to the liver or brain (stage IV), there was no cure (Vogelstein et al., 2013).

Knowledge of how cancers develop in polyps in the distal (descending, left side) colon reached the point where a large majority of malignant cancers that would arise in such polyps could be prevented by surgically removing the polyps. Cancer development in polyps that are not removed constituted the greatest risk of malignant colon cancer. Polyps were surmised to initiate due to a mutation in the APC gene, which normally functions to limit cell division in the colon's epithelium. A mutation in the APC gene inactivates this function and consequently allows cells to divide excessively, thereby producing a polyp (second panel from the left in Figure 6.8). Some of the cell's division controls however remains intact and puts a limit on the size to which the polyp could grow. The next step was found often to be a

mutation that over-activates a gene of the RAS family, which pushes cells to divide faster. That would cause the polyp to grow larger (third panel from the left in Figure 6.8). Another event that enhanced polyp growth was an inactivating mutation in the *TP53* gene (the topic of Chapter 32), a gene that normally stimulated DNA repair and caused division-control-defective cells to commit suicide by apoptosis.

This progression from early polyp to malignant cancer usually took many years, because mutations are rare events -- which was fortunate, because it allowed most colon cancers to be preventable by removing any polyps that may be seen during regular colonoscopies. A full-blown malignancy with distant metastases usually required several other mutations or gene function modifications; at that stage (stage IV), chemotherapy could prolong life, but was rarely curative.

Colon cancers, as well as most other types of cancer, only became truly malignant after mutations disturbed several genes that together normally kept a cell from growing wild. Figure 6.9 shows one of the methods that were used to display the pattern of gene mutations occurring in particular types of cancer (Vogelstein et al., 2013; Wood et al., 2007). We see that, in colon cancers, the genes for APC, KRAS, and TP53 stood out, although there were also many rare mutations that together could be important (Figure 6.9).

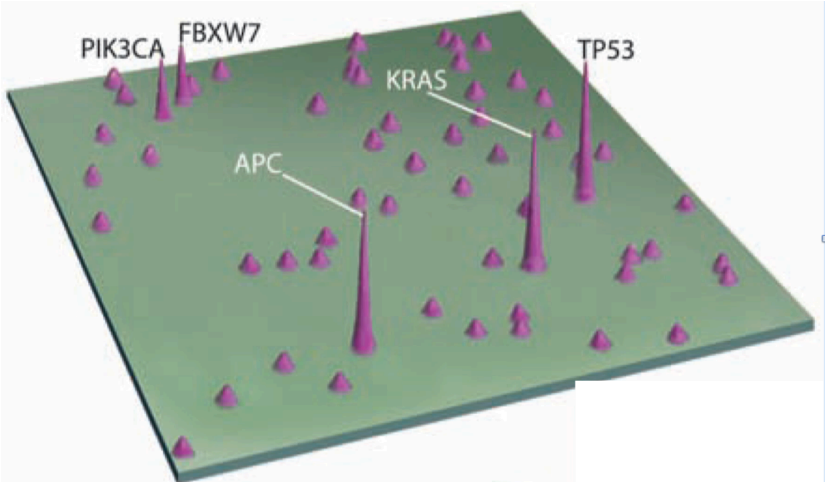


Figure 6.9. Genes that were found to be mutated in colon cancers. Three genes stood out: *TP53*, *KRAS*, and *APC*, and to a lesser degree *PIK3CA* and *FBXW7*. Less frequent mutations also occurred in many other genes. The gene mutation pattern differed from one type of cancer and another, although a few, in particular *TP53*, were found to be mutated in many or most cancer types. An *APC* gene mutation was common specifically in cancers arising in polyps in the descending colon. (The genes in this 2-dimensional landscape were arranged according their location on the chromosomes (Wood et al., 2007)).

How leucovorin enhances the anti-cancer action of 5FU.

Although leucovorin reversed the effects of methotrexate (see Chapter 5), in the case of 5FU leucovorin was found to enhance the drug's effect on its key target: thymidylate synthase, an enzyme required for production of thymidylate that is required for DNA synthesis (Figure 6.10).

The way in which 5FU and leucovorin together conspire to permanently block the thymidylate synthase enzyme was reported in classic papers by Dan Santi (Santi and McHenry, 1972) (Santi, 1980) (Figure 6.10). First of all, leucovorin easily converts to the active form: 5,10-methylene-tetrahydrofolate (CH_2-FH4). Normally, the thymidylate synthase enzyme binds both CH_2-FH4 and a uracil nucleotide and then transfers its CH_2 group as a methyl group to the uracil 5-position, thereby converting the uracil nucleotide to a thymine nucleotide that is essential for DNA synthesis.

5FU binds to the enzyme-CH_2-FH4 combination just as well as uracil does, but the transfer of the CH_2 to the uracil 5-position cannot proceed, because the fluorine atom tightly bound to that position. The enzyme becomes permanently trapped in a tight combination with CH_2-FH4 and 5FU -- and can no longer function of make the thymidine needed for DNA synthesis (Figure 6.10). It is beautiful how the tiny fluorine atom destroys the big enzyme.

Leucovorin increased the amount of CH_2-FH4 available, and thereby increased the rate or extent to which the thymidylate synthase enzyme binds 5FU. Leucovorin thus enhanced the trapping of the enzyme in the complex with 5FU. The result was that leucovorin enhanced the potency of 5FU in blocking DNA synthesis and thereby killing cancer cells. A limitation, however, was that, by the same process, leucovorin also increased the toxicity 5FU for rapidly dividing normal cells. Nevertheless, the net effect of leucovorin was to increase the anticancer effectiveness of 5FU (Figure 6.3).

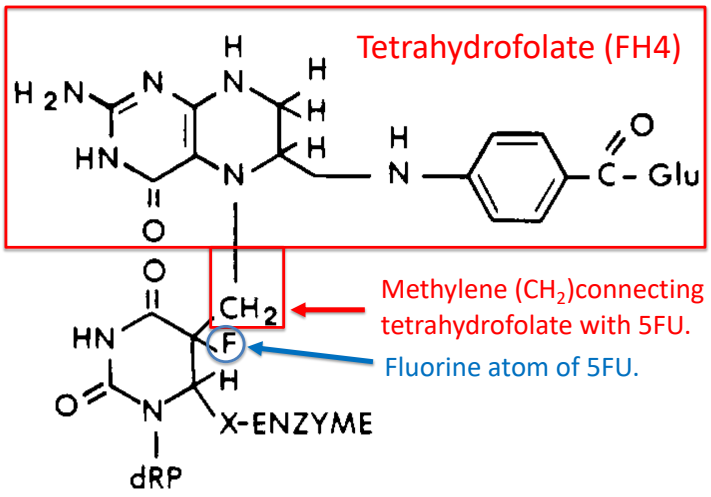


Figure 6.10. How 5FU, together with methylene-tetrahyrofolate (CH_2-FH4, an activated form of leucovorin) blocks the thymidylate synthase enzyme, as described by Dan Santi (the labels and lines in color were added to a figure copied from Santi's 1980 review paper (Santi, 1980)). Thymidylate synthase normally causes the CH_2 group of methylene-tetrahydrofolate to link to uridine on the way to converting the uridine to thymidine (as nucleotides) for DNA synthesis. The enzyme reacts similarly with 5FU, but the enzyme-5FU-CH_2-FH4 complex (shown in the Figure) then remains trapped because of the fluorine on position-5 and can proceed no further. That is how 5FU plus CH_2FH4 (or leucovorin, which converts to CH_2FH4), work together to block DNA synthesis. The thymidylate synthase enzyme binds, by way of a sulfhydryl group ('X' in the Figure), to the 6-position of the uracil, which puts the enzyme in position to carry out its normal work at the adjacent 5-position -- which however it cannot do when there is a fluorine atom there.

How 5-fluorouracil (5FU) kills cancer cells.

As described above, it is remarkable how much trouble a simple molecule like 5FU can cause to a cell. But to understand it better, we have to delve further into the complicated reaction pathways that 5FU gets into and messes up. First, in order to have any effects on a cell 5FU has to get into it.

How 5FU enters the cancer cell.

Since the cell needs a great deal of uracil for RNA and DNA syntheses, there are special transporter molecules that help move uracil rapidly in from outside the cell. Those same transporters allowed the cell to slurp up 5FU, leading to high concentrations of 5FU inside the cell. Cancer cells that grow rapidly need more

uracil and therefore make more transporter molecules, and these allow 5FU to enter rapidly. To the transporters, 5FU looks so much like normal uracil, that it moves 5FU into the cell as easily as it does uracil.

5FU stops the production of thymidylate for DNA synthesis.

Once inside the cell, 5-fluorouracil (5FU) enters uracil's metabolic pathways, because the enzymes that catalyze those reactions act on 5FU almost as well as on uracil (Figure 6.11). Again, the structure of 5FU is so much like uracil that the enzymes don't distinguish between them. It is much like a Trojan horse: it looks like a gift but turns out to be a poison (in German, Gift means Poison).

5FU becomes incorporated into DNA and RNA.

The most important action of 5FU was found to be inhibition of thymidylate synthase and consequent inhibition of DNA synthesis (Figure 6.11). The thymine-containing building blocks for DNA are depleted and uracil-containing units accumulate. Because there then is little thymidylate available, uracil and 5FU nucleotides, are mis-incorporated into newly synthesized DNA (Longley et al., 2003). Where there should be thymine in newly synthesized DNA, there often then is uracil or 5FU.

Thus, the scarcity of thymine units forces the DNA-synthesizing enzymes (DNA polymerases) to incorporate uracil or 5-fluorouracil (5FU) in place of thymine in DNA. Then, DNA repair enzymes come into play to remove the uracil and 5FU from the DNA, so that they could be replaced by thymine. But there is insufficient thymine nucleotide to replace the mis-incorporated uracil or 5FU with thymine efficiently! Hence, the DNA damage remains, and DNA functions are perturbed.

The metabolic scheme in Figure 6.11, shows how 5FU was thought (probably correctly) to become incorporated into DNA and RNA. 5FU first combines with ribose-phosphates to form 5FUDP (5FU-ribose-PP) and then 5FUTP (5FU-ribose-PPP). The latter (5FUTP) then is incorporated into RNA. For incorporation into DNA, on the other hand, the ribose part first has to be changed to deoxyribose, which entails removing the hydroxyl group from the 3’ position of ribose. This is accomplished by ribonucleotide reductase, which converts 5FU-ribose-PP to 5FU-deoxyribose-PP). Ribonucleotide reductase converts UDP (U-ribose-PP) to dUDP (U-deoxyribose-PP), which is an essential step for DNA synthesis. Because it is required for DNA synthesis, ribonucleotide reductase is itself an important anti-cancer drug target.

Hence 5FU makes its way into both RNA and DNA and thereby messed up functions in both realms (Akpinar et al., 2015). These deleterious effects, especially those

messing up DNA occur mainly in the phase of the cell cycle where DNA is being replicated, i.e., during S phase. That is why 5FU was often administered around the clock -- so as to give time for all the tumor cells to enter S phase, while the drug was still present and able to kill the cancer cells. 5FU was most effective against leukemias and lymphomas, most of whose cells were actively progressing around the cell division cycle.

Although much became known about what happens to 5FU in the cell, as described above, exactly how this complicated network of reactions added up to toxicity for the cell was not completely worked out (Huehls et al., 2016). 5FU kills cancer cells mainly by inhibiting thymine production (by inhibition of thymidylate synthase) and by becoming incorporated into DNA. Incorporation of 5FU into RNA adds to the toxicity and under some conditions could be the main factor causing cell death (Geng et al., 2011; Longley et al., 2003; Pettersen et al., 2011).

A closely related drug, 5FU-deoxyribose (5-fluorodeoxyuridine, FdUR) simplified the situation a bit by becoming incorporated mainly into DNA and less into RNA. But FdUR unfortunately did not seem to be much better than 5FU in cancer treatment experience.

For many cancers, the situation was complicated by the fact that only a fraction of the cancer cells is in the cell-division cycle at any one time. Great effort was therefore made to pin down the details of how a population of cancer cells progress around the cell cycle. Those efforts however did not have much impact on treatment of the major solid tumors, such as lung, breast, and colon cancers. Those cancers are slow growing and only a small fraction of the cancer cells is dividing.

For leukemias and lymphomas, however, most of whose cells are in the cycle, the detailed studies of the kinetics of the cell-division cycle did have a major impact. A major finding was that the time-scheduling of the treatments was critically important. Much better than continuous treatment with DNA synthesis-inhibiting drugs, was intermittent treatment with rest periods inserted to allow the bone marrow to recover. This anticancer drug scheduling was used successfully by Vincent DeVita and his colleagues in curing patients with Hodgkins lymphoma and is described in DeVita's book (DeVita Jr., 2015). (Although 5FU was not part of their 4-drug regimen, the principle was the same.)

What happens to DNA that has mis-incorporated 5FU and uracil in place of thymine.

Most of the mis-incorporated uracil and 5FU is rapidly removed by special DNA repair enzymes: uracil DNA glycosylases, as well other kinds of DNA glycosylases that could remove uracil and 5FU from DNA under different circumstances. (Huehls et al., 2016; Pettersen et al., 2011) (see Chapter 24). One of the DNA glycosylases

(MBD4) that helped to remove 5FU from DNA was specialized to remove mismatched thymine that was occasionally produced in certain places in the genome by spontaneous deamination of 5-methyl-cytosine at CpG sequences in gene promoters (Suzuki et al., 2016). Some mis-incorporated nucleotides may however remain and cause trouble.

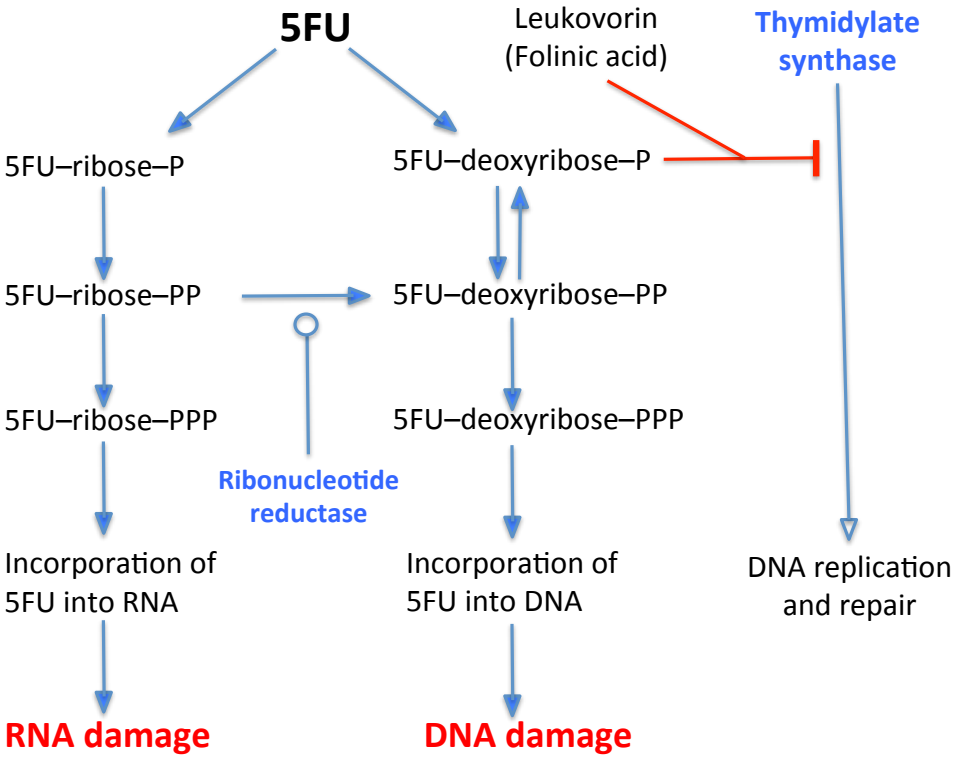


Figure 6.11. How 5-fluorouracil (5FU), a drug that simply has a fluorine atom added to uracil, has dramatic effects on the cell. 5FU enters the metabolic pathways that normally process uracil. One path leads to DNA, another to RNA. When 5FU enters these pathways, the result is DNA damage and disturbance of RNA function. In the DNA pathway, 5FU combines with deoxyribose, which is specific for DNA, whereas in the RNA pathway 5FU combines with ribose, which is specific for RNA. When 5FU combines with deoxyribose-P, it inhibits thymidylate synthase (Figure 6.10), the enzyme that adds a methyl group to a uracil unit to convert it to thymine, an essential for DNA synthesis. That is how 5FU exerts its major action: inhibition of DNA synthesis. Also required for DNA synthesis is the step where ribose is converted to deoxyribose, which is carried out by ribonucleotide reductase. which is itself an anticancer drug target. (P stands for phosphate. The technical terms are uridine mono- (di-, or tri-) phosphate; deoxyuridine mono- (di- or tri-) phosphate; similarly for the 5-fluoro compounds.)

The DNA mismatch repair system paradoxically helps kill cancer cells.

What happens to the 5FU that remains mis-incorporated in the DNA and what kind of trouble does it cause? 5FU in DNA was found to be recognized by DNA mismatch repair enzymes (enzymes that repair base-pairs that do not match); it was also found that, in order to give more time for this repair, these enzymes signal to the cell cycle control system that DNA synthesis should be delayed (Li et al., 2009).

You would think that DNA repair machinery should help cells to survive DNA damage. Cancers having competent DNA repair therefore ought to be relatively insensitive to DNA-damaging drugs. This is often true. However, in the case of certain kinds of DNA damage the opposite was found to be the case. We saw this paradoxical situation for the case of temozolomide, which damages DNA by adding a methyl group to the O6 position of guanine (Chapter 2). The DNA mismatch repair system seemed to cooperate with temozolomide in producing anticancer activity. This mismatch repair paradox occurred similarly for the case of 5FU: like O6-methyl-guanine, 5FU in DNA is recognized by the mismatch repair system, which fails in its attempts to repair the defect, leading to a persistent problem that eventually causes cell death (see Chapter 25). In both cases (5FU and temozolomide), the mismatch repair system goes into futile repair cycles and sends distress signals to tell the cell cycle control system to stop the cell from dividing. Eventually, it alerts the last-resort molecular decision makers that consign the cell to suicide by apoptosis (Li et al., 2009). That is how these complicated mechanisms were thought (probably correctly) to work. Thus, cancers that have normal DNA mismatch repair were paradoxically more sensitive to 5FU than cancers that lacked this repair machinery (Iwaizumi et al., 2011; Suzuki et al., 2016).

I will try to explain the reason for this strange state of affairs, where 5FU produced mismatched base pairs and the machinery intended to fix this defect instead helped to kill the cells. More details will be in a forthcoming chapter about DNA mismatch repair.

But first a quick reminder about the two parts of the colon that differ in whether the DNA mismatch repair system is intact or defective. Cancers in the proximal colon, which do not arise in polyps and do not respond to 5FU, usually had an inactivating mutation in one of the mismatch repair proteins. There are 4 proteins that make up this repair machinery, the most commonly mutated one being MLH1 (Figure 6.12) (Fleming et al., 2012) (Chapter 25).

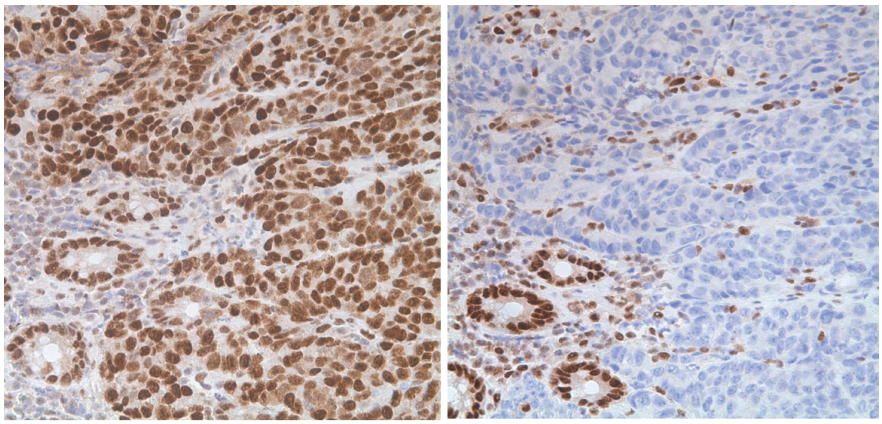


Figure 6.12. A DNA mismatch repair-deficient cancer in the proximal colon. Mismatch repair requires the function of 4 genes, among which are MLH1, which is frequently mutated in these cancers, and MSH6, which is rarely mutated. The cancer in this figure had an inactivating mutation of the MLH1 gene; therefore, the MLH1 protein was absent in the cancer (*right*). The MSH6 gene however was normal and its protein product was present in the tumor (*left*) (Fleming et al., 2012) (*Permission needed*.) (Fleming M, Ravula S, Tatishchev SF, Wang HL. Colorectal carcinoma: Pathologic aspects *Journal of Gastrointestinal Oncology*. 2012;3(3):153-173).

Why 5FU in DNA looks like a mismatch to the repair system.

The mismatch occurs, because 5FU can become ionized and then pairs better with guanine than its normal partner, adenine (Figure 6.13). The Fluorine atom's strong affinity for electrons attracts negative charge out of the ring, thereby facilitating ionization by loss of a hydrogen ion. As a result, the 5FU:adenine pair would be unstable and 5FU would preferentially pair with guanine, as further explained in the legend to Figure 6.13. (The ionization of 5FU is an equilibrium that depends on pH: the fraction of the time that the 5FU is ionized would be greater when the pH is higher.)

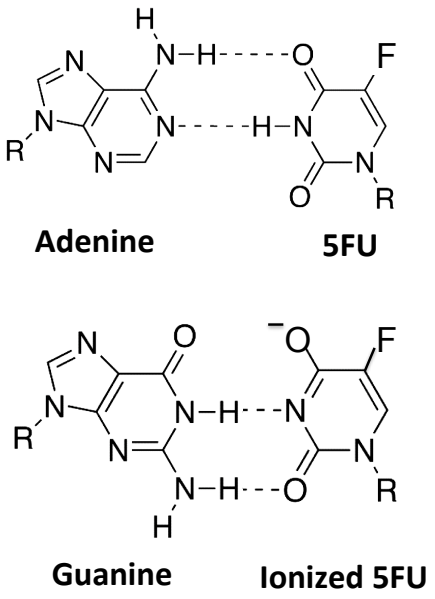


Figure 6.13. When 5-fluorouracil (5FU) has been incorporated into DNA, it can sometimes pair with guanine instead of adenine, which the mismatch repair machinery interprets as a mismatch. The pairing of 5FU with guanine occurs when the 5FU loses a hydrogen ion and becomes negatively charged, as shown in the lower part of the figure (Iwaizumi et al., 2011). 5FU becomes ionized, because the F pulls some of the electron charge out of the ring, allowing loss of a hydrogen from a ring nitrogen, which then can serve as an H-bond receptor, instead of H-bond donor.

Summary

The simplest anti-cancer drug, 5-fluorouracil (5FU) and one of the earliest to be developed, became the most important drug for the treatment of colon cancer. It was developed based on insightful intuition, which merely entailed addition of a fluorine atom to uracil, a nucleic acid building block. Despite this simple modification, 5-fluorouracil (5FU) disturbs several essential steps in DNA and RNA synthesis that cancer cells need in order to grow and multiply. In combination with surgery and other drugs, 5FU was able to cure a large fraction of colon cancer patients who had extensive local disease, but no distant metastases. It is remarkable how a drug simply made up of a normal uracil with a fluorine atom attached has a complicated mix of toxic actions and helps in the therapy of certain cases of colon cancer, as well as other cancers.

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