Biochemistry—Not Oncogenes—May Demystify and Defeat Cancer

Jay Kulsh

ABSTRACT

The presence of mutated genes strongly correlates with the incidence of cancer. Decades of research, however, has not yielded any specific causative gene or set of genes for the vast majority of cancers. The Cancer Genome Atlas program was supposed to provide clarity, but it only gave much more data without any accompanying insight into how the disease begins and progresses. It may be time to notice that epidemiological studies consistently show that the environment, not genes, has the principal role in causing cancer. Since carcinogenic chemicals in our food, drink, air, and water are the primary culprits, we need to look at the biochemistry of cancer, with a focus on enzymes that invariably facilitate transformations in a cell. In particular, attention should be paid to the rate-limiting enzyme in DNA synthesis, ribonucleotide reductase (RnR), whose activity is tightly linked to tumor growth. Besides circumstantial evidence that cancer is induced at this enzyme’s vulnerable free-radical-containing active site by various carcinogens, its role in initiating retinoblastoma and human papillomavirus (HPV)-related cervical cancers has been well documented in recent years. Blocking the activity of malignant RnR is a certain way to arrest cancer.

Keywords: Carcinogenesis; Cancer treatment; Cervical cancer; DNA synthesis; Free radical; Retinoblastoma; Ribonucleotide reductase (RnR); Somatic mutation theory (SMT)
Key Summary Points

For the vast majority of cancers, the genetic theory is inadequate in explaining initiation or progression of the disease.

Many “tumor suppressor genes” have been identified but “cancer-causing oncogenes” remain elusive.

Since carcinogenic chemicals cause most cancers, we examined cancer biochemistry, with a focus on enzymes that invariably mediate cellular transformations.

Hyperactivity of the ribonucleotide reductase (RnR) enzyme is implicated in retinoblastoma and HPV cancers; furthermore, its active site is vulnerable to carcinogens—making it a likely epicenter of cancer.

The free-radical-containing active site of RnR can be disabled biophysically, resulting in a non-toxic cancer treatment.

In 2005, at the onset of The Cancer Genome Atlas (TCGA) program, Dr. Eric C. Lander, director of Broad Institute of MIT and Harvard, declared in New York Times, “Knowing the defects of the cancer cell points you to the Achilles’ heel of tumors” [1].

At Cancer World 2013, Nobel laureate James Watson opined, “We can carry on and sequence every piece of DNA that ever existed, but I don’t think we will find any Achilles heels [of cancer]” [2].

INTRODUCTION—CANCER WAR NOT WON

The year 2021 marked the 50th anniversary of the declaration of “war on cancer” by US President Nixon. He was inspired by President Kennedy’s declared goal in 1961 to land a man on the Moon by the end of the decade—which had come true, to international acclaim. However, progress in the field of cancer has been quite limited, despite plentiful funding over the last five decades.

The overall survival rates have improved, to varying degrees, for all cancers, primarily due to early detection and intervention. Preventative measures, such as the reduced use of tobacco products, have also lowered the incidences of lung cancers [3]. However, effective treatment remains out of reach for most cancers.

Common reasons given for this slow progress is that cancer is a very complex disease—and that it may not be one but many diseases. However, there is another possibility, raised as early as 1994 by Scientific American magazine: “Have the researchers and clinicians been barking up the wrong trees...?” [4]. Then as well as now, the “wrong trees” referenced in this statement, would all be gene related, since the vast majority of cancer research projects, during the last five decades, have focused on the genetic aspect of the disease. Could this approach, this emphasis on genes, be wrong to understand and tackle cancer?

There is growing evidence that this in fact may be the case [5–7]. The close correlation between mutagenesis and cancer may not be causative in nature. Mutated genes may simply be a consequence of carcinogenesis, initiated elsewhere upstream. If so, then the genes-centered rationale may be hampering researchers in their fight against cancer.

This possibility is explored here. Various aspects of the genetic theory of cancer are examined against experimental evidence gathered over the past half-century. Next, those studies are parsed that ascertain whether the locus of tumorigenesis is in the nucleoplasm or cytoplasm. The article then discusses an alternative mechanism for the induction of cancer where a free-radical-based enzyme plays a central role; this may explain many hitherto mystifying aspects of the disease. Finally, a way to control cancer by biophysical means is cited.

This article is based on previously conducted studies and does not contain any new studies.
with human participants or animals performed by the author.

**ESSENCE OF THE GENE-CENTRIC VIEW OF CANCER**

Per the prevailing genetic model, cancer is caused by mutation of genes, which are either inherited or acquired.

- **An “inherited gene mutation”** is present in the egg or sperm cell of the parents. In rare instances, post-zygotic de novo mutation can occur during early embryonic development. Such germline mutation is in every cell and so may be passed on to the next generation.

- **An “acquired (somatic) mutation”** is not present at birth, but is acquired sometime later. Acquired mutations are much more common and most cancers are caused by them. This somatic mutation theory (SMT) has become the dominant paradigm in cancer research [8].

Per the SMT, a multistep process—initiation, promotion, and progression—of random mutations in some key genes leads to cancer. These key genes are either oncogenes or tumor suppressor genes.

An oncogene results from mutation of proto-oncogenes. The latter are healthy normal genes that help cells grow. Oncogenes can become permanently activated causing cells to grow out of control. Too many copies of a proto-oncogene are also called “oncogene.”

Tumor-suppressor genes are normal genes that slow down cell division, repair DNA mistakes, or tell cells when to die (apoptosis). When these genes are mutated or defective, cells can grow out of control, leading to cancer [8].

Sustained proliferative signaling leads to nuclear genomic instability, which underlies various hallmarks of cancer.

(Further elaborations of this model—epigenetic alterations and the classification of mutations as drivers or passengers—are discussed elsewhere in the article.)

**Core Deficiencies of the Genetic Theory of Cancer**

**Carcinogens Relegated to the Side**

Only 5–10% of all cancers are attributable to genetic defects. The remaining 90–95% have their roots in the environment and lifestyle [9].

In other words, most cancers are caused by chemical carcinogens, be they in tobacco products, food additives, pesticides, or environmental pollutants. But the genetic theory of cancer minimizes their significance. Cancer risk is typically discussed in terms of the importance of genes: high-, moderate-, and low-penetrance [10]. In the least important last group are two kinds of genes: DNA-repair and xenobiotic metabolizing genes [11, 12]. The word “xenobiotic” means “foreign to the body”—and only under this category carcinogens are mentioned, almost as an afterthought.

Chemical carcinogens are, of course, foreign to the body, but they must be put front and center when we are trying to understand cancer.

**No Mechanism for Selective Mutations**

In our body, exogenous chemicals go through one or more metabolic pathways—the majority mediated by the versatile cytochrome P450 enzymes—which may result in excretable compounds, or produce metabolites that are reactive and toxic [13]. In the case of asbestos particles, as well as UV light and ionizing radiation, reactive oxygen species (ROS) are produced [14–16]. How can these myriad reactive molecules selectively attack only those genes which are involved in cell duplication (chromosome segregation, mitosis, cytokinesis), DNA repair, or cell death (apoptosis)? Such genes are a small minority—less than 10%—of the protein-coding genome [17–20], and from the perspective of these relatively tiny chemical compounds, the DNA strand of one gene is no different than that of any other gene. (Carcinogens are usually not known to cause other kinds of genetic damage to the cell, which would indicate genome-wide assault.) No mechanism is known by which carcinogens, or their metabolic products—or old age—might impair only those genes—a superset of proto-
oncogenes and tumor suppressor genes—which are involved in cell division, DNA repair, and apoptosis.

The Immediate Cause of Unrestrained Cell Growth Unknown

Even if we ascribe carcinogens or their transformed products some mysterious power to locate the relevant proto-oncogenes to damage them—or attribute such damage to age-related “bad luck” random deteriorations—how would these point mutations trigger uncontrolled cell growth? Normally, a defect in an entity results in its functioning at a reduced level or not functioning at all. (That is what happens when “tumor suppressor genes” are mutated.) Despite decades of intensive hunt, no cancer-causing genes (oncogenes) have been identified that are necessary and sufficient to cause cancer.

Over the last two decades, modifications in epigenetic regulators such as CpG island methylation, histone acetylation, and microRNA (miRNA)-associated silencing have been introduced as possible pathways in tumorigenesis [21]. The mechanisms of such alterations do seemingly account for the role of xenobiotics in our environment [22]. However, the immediate cause of uncontrolled cell proliferation remains as elusive as ever.

In brief, the gene-based theory of cancer fails to answer certain basic questions about the process of carcinogenesis. The raison d’être for the concept of “oncogenes” is correlation or the presence of mutated genes at the site of cancerous growth. Could these mutated genes be end products of malignant growth, similar to dead and wounded soldiers at the end of a war? Even healthy soldiers do not cause war since they are simply following orders from higher ups. Could genes be no different, merely responding to biochemical signals or cues?

The next two sections evaluate in detail the evidence for the role of genes in causing inherited and noninherited cancers.

In Hereditary and Familial Cancers, Few Oncogenes Identified

Having certain inherited genetic mutations may increase a person’s risk of developing cancer, but it does not necessarily mean that person will get cancer. Such medical conditions are usually called “syndromes.” The most common hereditary cancer susceptibility syndromes are Lynch syndrome, Li-Fraumeni syndrome, Von Hippel-Lindau syndrome, Familial Adenomatous Polyposis (FAP) and Cowden syndrome. The cancer risk can be passed from generation to generation in a family, though some de novo mutations are also seen without a family history. They all follow an autosomal dominant inheritance pattern, wherein a single copy of the specific mutation is enough to predispose a person to the disease [23, 24].

In all these syndromes, tumor suppressor genes in germline mutations are the culprit [25].

The tumor suppressor genes (BRCA1 and BRCA2) are also implicated in familial breast and ovarian cancers. They increase susceptibility to these cancers and are passed down through a family in an autosomal dominant manner. Only 5–10% of breast cancers fall in this “hereditary” category; most such cancers occur sporadically in people with no family history [25].

A tumor suppressor gene is also involved in retinoblastoma, which is a historic case. The mutation or deletion in its RB1 gene was the earliest one identified, in 1971, and gave a boost to the gene-based view of cancer [26]. This childhood disease is unique and not called a syndrome since the nonfunctional RB1 gene does not simply predispose but directly causes cancer of the retina when both alleles are defective or missing. Approximately one out of three cases are due to germline mutation, usually acquired during early development in the womb, and rarely inherited from a parent. The majority of retinoblastomas are noncongenital and develop in only one eye [24, 25].

On the other hand, mutations in the proto-oncogenes have been identified in only two types of such cancers: RET and MYCN/ALK genes in multiple endocrine neoplasia type 2 (MEN2) and neuroblastoma, respectively. MEN2
occurs via a germline mutation, linked to a high lifetime risk of developing medullary thyroid cancer, but some sporadic mutations are needed for the onset of the disease [25, 27]. Neuroblastoma is a cancer of nerve cells and occurs mainly in children, but a family history of the disease is found in only 1–2% of cases [25].

Thus, in cancers with hereditary components, time and again, the genes associated are tumor suppressor genes. Only rarely do we find proto-oncogenes, and when we do, some external noninherited factors are involved in turning them into bad cancer-causing oncogenes. (In the next section, oncogenes are discussed in detail.)

In Somatic Cancers, Many Oncogenes, but no Clear Causative Role

Despite extensive search, over the last many decades, for proliferation-inducing “oncogenes” and mutated “tumor suppressor genes,” no meaningful pattern has emerged for any type of noninherited (somatic) cancer.

The findings of various studies show several inconsistencies [28]. For example:

- The same tumor may contain many distinct foci, with different subpopulation of mutations in each [29].
- Mutations may vary dramatically from one stage of tumor progression to another [30].
- Metastasized tumors often have different mutations than the primary tumor [31].
- For the same type of cancer, mutated genes are often different and random in different individuals [30].
- The presence of mutations differs from country to country. In non-small cell lung cancer tumors, mutations found in 15 out of 58 Japanese patients were there in only 1 out of 61 US patients with the same cancer [32].

Thus, mutated genes in a tumor are varied and random at all levels, making any functional and causal interpretation very difficult. As if every cancer cell is a unique experiment in nature, having its own mutational signature, reflective of its distinct lineage history within the evolving neoplasm [33].

Unsurprisingly, the majority of representative “high-quality” cancer research papers are unreproducible. Scientists at Amgen could replicate findings in only 6 (11%) of 53 such published papers [34].

Epidemiological Evidence against the Genetic Basis of Cancer

The genetic theory of cancer explains the increase in human cancers in post-industrial societies by pointing to the significant lengthening of our lifespan. It posits that age-related molecular and physiological deteriorations may act in concert to promote cancer. The combined pathogenetic effects of accumulated “bad luck” mutations, increased epigenetic dysregulation, telomere dysfunction, reduced DNA repair capacity, and altered stromal milieu—all take their toll on cells [35, 36]. In short, according to the gene-based view, cancer is largely a disease of old age.

But epidemiological studies often undermine this assertion. An editorial in the New England Journal of Medicine stated: “Geographic differences, trends over time in the risk of cancer, and detailed studies of migrant populations overwhelmingly implicate environmental exposures as major causal factors and often identify the responsible carcinogens (e.g., tobacco, alcohol, radiation, occupational toxins, infections, diet, drugs). From this work has come the widely accepted estimate that 80–90 percent of human cancer is due to environmental factors” [37].

Data analysis of childhood cancers from 19 European countries showed an annual increase of 2% in infant cancer from 1978 to 1997. Incidence rates of many, but not all, cancers in children and adolescents were rising [38].

Similarly, an epidemiological study, funded by the National Cancer Institute (NCI), alarmingly found that the incidence of cancer in the “15–29 years” age group increased steadily between 1975 and 2000 [39].

A recent study has estimated the cancer burden attributable to 13 occupational carcinogens across 195 countries in the years 1990–2017. It found that, except in the most advanced countries (top one-fifth), cancer
deaths were higher in the “50–69 years” age group compared with the “older than 70 years” group; people aged “15–49 years” also died from cancer [40].

Such findings cannot be reconciled with the view that cancer is essentially a disease of old age.

**Epidemiology Gold Standard—the Study of Twins**

Studies of monozygotic (identical) and dizygotic twins are unsurpassed in distinguishing genetic from environmental traits.

A study published in Lancet reported that if one member of a pair of identical twins develops acute leukemia in childhood, the chances of leukemia developing in the other twin are no higher than 20%. The concordance decreases with increasing age of onset of leukemia, being highest during the first year of life and low after 7 years of age [41].

Among 432 sets of twins, when at least one was affected by Hodgkin’s disease, none of the 187 pairs of dizygotic twins became concordant for Hodgkin’s disease, and only 10 of the 179 pairs of monozygotic twins did [42].

In a 2000 study, the data on 44,788 pairs of twins in Sweden, Denmark, and Finland showed that inherited genetic factors make a minor contribution to susceptibility to most types of cancers. The environment had the principal role in causing sporadic cancer [43]. This study was updated and expanded in 2016 to include Norway and many more cancer types [44]. The data showed an excess cancer risk in twins whose co-twin was diagnosed with cancer: an absolute 5% and 14% higher in same-sex dizygotic and monozygotic pairs, respectively, by the age of 100 years. Notably, more than two-thirds of these twins were diagnosed with a different malignancy, which can only partially be explained by pleiotropy. Furthermore, the median time between cancer diagnoses in concordant pairs of twins was fairly long: 4–15 years. These results suggest the majority role for non-genetic factors such as environment and lifestyle.

Such studies contradict the view that genes play a preeminent role in incidences of cancer.

**The Cancer Genome Atlas (TCGA) Program—More Data, No Clarity**

In 2006, a landmark cancer genomics program, The Cancer Genome Atlas (TCGA), was launched by the National Cancer Institute (NCI), sequencing over 20,000 primary cancers spanning 33 cancer types [45].

The goal of the program was to generate sufficient data so that some knowledge gaps are filled and the landscape of cancer genetics becomes comprehensible. However, these aims were not achieved. No recognizable patterns emerged. No synergistic accounts could be provided. Observed genetic mutations remain heterogeneous or diverse as well as random and complex [46, 47]. We do have an ever increasing collection of genetic changes associated with cancer that need cataloging [30].

The enormous amount of data has not yielded any specific gene mutation—or any combination of mutations—that is necessary, let alone adequate, to initiate the transition from one stage of malignant neoplastic progression to the next. A considerable disconnect is seen between nominal “oncogenic mutations” and cancer phenotypes [48].

There is a sense that accumulated data may be overwhelming the researchers’ abilities of interpretation [33]. However, a small effort is made by putting all the mutations in two groups: drivers and passengers. Driver mutations are causal, whereas passenger mutations occur by chance. Such ad hoc elaborations have been seen, by some critics, as akin to the use of epicycles in pre-Copernican astronomy [49]. The futility of such attempts becomes obvious when driver mutations are found in benign and premalignant conditions, occasionally at higher frequencies than in their malignant counterparts [50]. The concept of the cancer-inducing “oncogene” is looking increasingly like a phantom—and SMT no more than an epiphenomenon. A new paradigm is, therefore, called for [5, 6, 51].
SOURCE OF CARCINOGENESIS MAYBE OUTSIDE THE NUCLEUS, AWAY FROM GENES

As early as 1975, it was reported that the carcinoma cells, after introduction into normal blastocysts, can give rise to a large variety of normal tissues—mostly strikingly, sperms—in mosaic mice, thereby showing that tumorigenesis may not involve damage to genes, but only aberrations of gene expression [52].

A study in 1987 showed that when cells are reconstituted by fusing karyoplasts from malignant cells with the cytoplast of normal cells, the tumorigenic phenotype was extinguished [53]. In 1988, the same authors reported that in cells derived by fusion of cytoplasts from malignant cells with karyoplasts of normal cells, tumors were seen in 97% of the animals injected [54]. Figure 1 is a visual depiction of their findings:

At the end of that 1988 article, one can see:

EDITOR'S STATEMENT
This is the first description of cytoplasmic mediation of tumorigenesis. There is a clear indication that cytoplasmic elements play a role in the expression of the malignant phenotype.

This addendum was highly unusual, and for a good reason. It was a clear demonstration that genes may play little or no role in carcinogenesis—contrary to the prevailing view of cancer as a genetic disease. Even after 34 years, such evidence is being ignored. It is hard to shake the belief of the vast majority of cancer researchers who cannot take their eyes off genes [6].

Subsequent researchers have confirmed this phenomenon. For example, in 2003, it was reported that blastocysts derived from medulloblastoma nuclei form embryos with typical cell layers, showing normal patterns of tissue differentiation [55]. Interestingly, these results were interpreted as “epigenetics reprogramming abrogating tumorigenic phenotype,” which is a stretch since even the most ardent proponents of the “epigenetics model of carcinogenesis” state that genetic mutations are secondary “critical by-products,” not absent [21]. Therefore, how could the transfer of nuclei make those genetic mutations go away?

A more plausible explanation was hinted at earlier: the root of cancer may not be malfunctioning genes, but some biochemical processes in the cytoplasm that may be triggering unrestricted cell growth. The remainder of this article will delve into the biochemistry of cancer.

Looking for the Origin of Cancer in the Cytoplasm’s Biochemistry

Due to the conceptual limitations of the cancer gene mutation theory, and the accumulated evidence against it—as described in the previous sections—researchers have started looking outside the nucleus to unravel the mechanisms underlying cancer development.

In recent years, most of the attention has been paid to the mitochondria. The century-old Warburg hypothesis has been revived, which posits that mitochondrial dysfunction is the root cause of cancer, resulting in glucose fermentation even when enough oxygen is present. In the updated version, a plethora of
random somatic mutations in tumors are seen as downstream effects of insufficient respiration with compensatory fermentation [56]. Examples of potent mutagens such as ROS are given, which are produced in damaged mitochondria.

Unfortunately, this carcinogenesis theory cannot explain what attracts carcinogens (or their metabolic products) to mitochondria and why uncontrolled multiplication of cells occurs when there is mitochondrial malfunction. Therefore, this theory is as weak as the genetic theory in explaining some basic facts about carcinogenesis.

Among those frustrated at the glacial pace of progress on the cancer front is one of the founding fathers of molecular biology, Dr. James Watson, who spent three decades exploring the genetic aspects of cancer as director of Cold Spring Harbor Laboratory, NY. On May 12 2016, in the New York Times, he stated:

... locating the genes that cause cancer has been “remarkably unhelpful”—the belief that sequencing your DNA is going to extend your life is “a cruel illusion”. If he were going into cancer research today, he would study biochemistry rather than molecular biology [57].

To seek mechanisms of cancer causation in biochemistry is far from counter intuitive. After all, most cancers are caused by toxic chemicals and the earliest drugs designed to treat cancer, just after World War II, were based on the knowledge of biochemistry [58]. Most of the chemotherapeutic drugs, synthesized ever since, often target some biochemical pathway implicated in cancer.

To fully understand the biochemistry of cancer, we must look at the activity of various enzymes involved in cancerous proliferation, as virtually all transformations in a biological cell are mediated by one or more enzymes.

**PIVOTAL ENZYME IN CANCER GROWTH MAY DEMYSTIFY CANCER**

Ribonucleotide reductase (RnR) converts the building blocks of RNA into the building blocks of DNA, as shown in Fig. 2. Besides catalyzing the de novo production of DNA precursors, RnR is also involved in DNA repair. It is the rate-limiting enzyme for DNA synthesis, without which no cell can replicate, and no cancer can grow [59].

The activities of prominent enzymes in healthy and cancerous tissues are compared in a table (as Fig. 3). In a growing tumor, the activity of the enzyme ribonucleotide reductase jumps up exponentially, much more than that of any other enzyme—almost eight-fold higher than even DNA polymerase [60].

The enzyme RnR consists of two dissimilar subunits, proteins RRM1 and RRM2 (sometimes written as RnR-α and RnR-β). The larger RRM1 is a dimer and binds substrates as well as allosteric effectors. The smaller RRM2 is also a dimer, and contains a tyrosyl free radical which is stabilized by an adjacent oxo-bridged binuclear iron center [61].

*Fig. 2* Ribonucleotide reductase (RnR) converts the building blocks of RNA into those of DNA
Comparison of activities of pyrimidine- and DNA-synthentic and -catabolic enzymes in liver and in rapidly growing hepatoma

Data are expressed as specific activity and as percentages of the normal liver values. In calculations, the values 200 mg of protein per g, wet weight, of tissue for homogenates and 80 mg of protein per g for supernatant fluids were used. Enzymic activities were those determined in this laboratory and in other centers.

| Enzymes                       | EC no. | Normal liver (pmol/hr/mg protein) | Rapidly growing hepatoma 3683F (%) of liver |
|-------------------------------|--------|----------------------------------|---------------------------------------------|
| Anabolic enzymes              |        |                                  |                                             |
| Ribonucleotide reductase      | 1.17.4.1 | 23                              | 18,348                                      |
| DNA polymerase                | 2.7.7.7  | 56                              | 5,806                                       |
| dTMP synthase                 | 2.1.1.b  | 180                             | 2,860                                       |
| dTMP kinase                   | 2.7.4.9  | 420                             | 7,000                                       |
| Deoxycytidine kinase          | 2.7.1.74 | 800                             | 1,400                                       |
| Thymidine kinase              | 2.7.1.21 | 900                             | 3,920                                       |
| CTP synthetase                | 6.3.4.2  | 5,500                           | 1,122                                       |
| Carbanomoyl-phosphate synthetase II | 2.7.2.9  | 10,000                          | 950                                         |
| dCMP deaminase                |        | 12,000                          | 750                                         |
| Uracil phosphoribosyltransferase | 2.4.2.9  | 19,000                          | 760                                         |
| Ornithine-5’-monophosphate decarboxylase | 4.1.1.23 | 34,000                          | 889                                         |
| Orotate phosphoribosyltransferase | 2.4.2.10 | 47,000                          | 599                                         |
| Uridine phosphorylase         | 2.4.2.3  | 164,000                         | 671                                         |
| Uridine-cytidine kinase       | 2.7.1.48 | 156,000                         | 694                                         |
| Dihydroorotase                | 3.5.2.3  | 246,000                         | 418                                         |
| Aparatate carbanomoyltransferase | 2.1.3.2  | 448,000                         | 706                                         |
| UDP kinase                    | 2.7.4.6  | 444,000,000                     | 298                                         |
| Catabolic enzymes             |        |                                  |                                             |
| Dihydrouracil dehydrogenase   | 1.3.1.2  | 26,000                          | 9                                           |
| β- Ureidopropionase           | 3.5.1.6  | 144,000                         |                                             |
| Thymidine phosphorylase       | 2.4.2.4  | 234,000                         | 31                                          |
| Dihydropyrimidinase           | 3.5.2.2  | 276,000                         |                                             |

Fig. 3 Reused with permission from Weber (1983) [60]

\[
\text{Tyr} - \text{O}^\bullet \rightarrow - - \text{(Fe}^{3+} - \text{O} - \text{Fe}^{3+})
\]

The free radical at the core of the RRM2 subunit is essential for any enzymatic action. The active RnR enzyme, a hetero-oligomer, is often depicted as \(\alpha_2\beta_2\), as shown in Fig. 4.

The overall RnR activity is controlled by the \(\alpha_2\) activity A-site through interactions with deoxyadenosine triphosphate (dATP); inhibitory or ATP (adenosine triphosphate); stimulatory. Nucleoside triphosphates (NDP) reduction to deoxynucleoside triphosphate (dNDP) takes place in the catalytic C-site of \(\alpha_2\) and involves the unpaired electron, which is initially localized in \(\beta_2\) as a diferric–\(\text{Y}^\bullet\) cofactor (the “metallocofactor”). The required relocation of the free radical involves a proton-coupled electron transfer chain to generate a transient thyl radical (\(\text{S}^\bullet\)) in the \(\alpha_2\) C-site, which initiates the reduction of the NDP ribose ring, thereby generating dNDP and resulting in disulfide bond formation there. The oxidized cysteines of \(\alpha_2\)
are re-reduced by thioredoxin or glutaredoxin, facilitating turnover. [62]

The levels and activity of RnR are tightly regulated via multilayered mechanisms that involve intricate interplay between gene expression, cell cycle checkpoints, subunit oligomerization, and proteolysis, together with allosteric effects via two allosteric sites, the specificity site (S-site) and aforementioned A-site [59]. Exquisite controls of RnR activity are necessary to optimally regulate cell multiplication.

Obviously, this critical enzyme for cell division is known to impact cancer susceptibility [62]. Lately, the RnR-complex has been called “oncogenic” in the sense of proliferation inducing (no genes are there, only unique proteins) [63]. The next small step needs to be taken to view this enzyme as the site where, in all probability, cancer is initiated.

The RnR-based model of carcinogenesis proposes that the RRM2 subunit of this enzyme, containing a diferric-tyrosyl-radical active site, is the primary, and perhaps the only, target of all types of carcinogenic chemicals. Various characteristics of tumor cells—anchorage independence, dedifferentiation, metastasis, angiogenesis, genetic aberrations, chromosomal anomalies, genomic instability, etc.—all result from the cascade of events that is initiated at the RRM2 subunit by adverse stimuli.

It is noteworthy that RnR resides in the cytosol. The deoxynucleotides produced by it diffuse into the nucleus or are transported into the mitochondria [64]. Thus, this model is in accordance with the observations depicted in Fig. 1.

The mechanism of initiation of cancer at the RRM2 subunit of RnR in a variety of malignant neoplastic transformations is elucidated below (the first two sections are based on circumstantial evidence while direct experimental proof exists for the remaining two):

### Chemicals

Carcinogenic chemicals, thought to be culpable in a large number of cancer cases, fall into two broad categories: direct acting and indirect acting. Direct carcinogens, of which there are only a few, e.g., dimethyl sulfate, are reactive electrophiles, i.e., they seek out and react with negatively charged centers in other compounds. Indirect carcinogens such as highly inert polycyclic aromatic hydrocarbons, can attain even more reactive electrophilic centers by going through any of the various metabolic oxidative pathways in the body, involving powerful cytochrome P-450 enzymes [13].

In addition to being or becoming electrophilic, chemical carcinogens are, in general, hydrophobic in nature. Molecules with these characteristics are especially suited to access the RnR active site since Glu/Asp residues around ferric ions constitute a negatively charged environment [62, 65], and the tyrosyl free radical is located in a hydrophobic pocket [62, 66].
According to this mechanism of cancer induction, carcinogenic chemicals and/or their metabolic products, are attracted to the electromagnetically charged active site of RnR, and once there, would disturb the finely tuned rhythmic controls in place. The accumulation of such miscreant/irritant molecules may go on for years, until a threshold is crossed, resulting in persistent overstimulation. A chain of events is then set in motion culminating in the production of outlaw tumor cells. Responding to the “non-self” antigens on the surface of these cells, the immune system would eliminate them. However, over a period of time ("the latency period"), weakly antigenic cancer cells, capable of evading immune surveillance, would evolve—and cancerous growth gains a foothold.

Re-evaluating the Chemical Structure of a Compound for Carcinogenicity

The currently established tool Quantitative Structure–Activity Relationship (QSAR) to test the carcinogenicity potential of a compound is predicated on the affinity of its electrophilic center to nucleophilic moieties in the four bases of DNA [67]. However, such an approach is highly inadequate, if not problematic, because of its lack of any specificity. The human genome consists of billions of bases—and most are not part of any protein-coding genes. As mentioned in “No mechanism for selective mutations” section above to a carcinogenic molecule, one section of the DNA strand is no different from any other, and therefore, it can cause genetic damage or mutations only in an entirely random fashion. How can such a process lead to uncontrolled cell growth—and at the same time, cause no other harm?

This shortcoming may be overcome if we view the diferric–tyrosyl radical-containing active site of RnR as the target-site of carcinogens. The vulnerability of this site should be different for different exogenous compounds (or their metabolic products), depending on their structures—quite likely in line with their carcinogenicity.

Asbestos and Radiations

Extremely fine asbestos fibers are known to cause malignant tumor growth after a latency period of 20–40 years. After uptake by lung cells, phagocytic cells that engulf asbestos fibers produce large amounts of ROS and other free radicals due to their inability to digest the fibers. This model provides for these oxygen radicals a singularly sensitive target-site to initiate, albeit unwarranted, cell proliferation—in a manner similar to carcinogenic chemicals. A slow struggle with the immune system would then commence, which may be lost only after a few decades. It is notable that epidemiological studies indicate that iron-containing asbestos fibers appear more carcinogenic [14].

The cases of UV light and ionizing radiation are very similar, since both generate elevated levels of ROS in the human body [15, 16]. However, their latency periods are considerably shorter.

HPV-Related Cervical Cancers

High-risk human papilloma viruses (HPVs) are causal agents for human cervical cancers. The viral proliferation activity is completely dependent on its genes E6 and E7, the latter of which is more potent and induces upregulation of the RRM2 subunit of RnR in the host [68]. Exogenous activation of RnR, which was initially meant to help make copies of only the invading virus, over time ("latency period") starts building unneeded copies of the cells of the human host.

Diferric–tyrosyl radical-containing RRM2 has now become a molecular marker for the diagnosis and clinical outcomes of cervical cancer [69].

Retinoblastoma

While studying the mechanism of cancer causation by HPVs, researchers discovered that the E7 protein of the virus targets and inactivates the retinoblastoma gene (RB) [70]. This results in the release of active E2F transcription factors, which then bind to the RRM2 promoter region,
putting RnR in overdrive—and causing the improper proliferation of cells [68]. These findings make it possible to understand why non-functional or missing RB genes in infants are responsible for retinal cancer, mediated by unchecked activity of RnR.

Thus, we have seen that in various types of cancer, persistent over-stimulation or over-expression of the radical-containing RRM2 subunit of the enzyme RnR may be the trigger that sends the entire elegant cell-division machinery spinning out of control. Radicals are being recognized for their important role in cell signaling [71] and a strong case has been made to view the RRM2 subunit as an “oncoprotein”—in addition to its being part of a critical enzyme [72]. Unlike a normal cell division, in this aberrant process, allosteric controls at specificity (S-) and activity (A-) sites of RRM1 would be poorly implemented, resulting in unbalanced and elevated dNTP levels. This loss of homeostasis of DNA building blocks would lead to mutations and genome instability—hallmarks of cancer [73].

This RnR-based model of carcinogenesis answers the hitherto unanswerable questions about cancer: What is the immediate cause of unrestrained cell growth? How do carcinogens cause cell replication to go out of control?

**DISABLING MALIGNANT RIBONUCLEOTIDE REDUCTASE WILL DEFEAT CANCER**

Whether or not cancer is initiated, in all cases, at the enzyme ribonucleotide reductase (RnR), it is beyond dispute that if you stop RnR, you stop cancer. Therefore, this enzyme is considered a classical target for cancer therapeutics [72, 74].

Chemotherapeutic compounds aiming to block this enzyme have been synthesized since the early 1970s [75]. They fall in three broad categories: (1) nucleoside analogs targeting allosteric sites of RRM1 [62, 76], (2) compounds targeting α/β interface to disrupt assembly of subunits [77], and (3) iron chelators or radical quenchers targeting diferric–Y• cofactor of RRM2 [62, 78].

Unfortunately, these drugs have limited potency and produce toxic side effects. Also, malignant cells become resistant to them over time. Despite this, several RnR inhibitors approved by the US Food and Drug Administration are used clinically to treat various forms of cancer [62].

A novel way of inhibiting this enzyme is suggested by the fact that the free radical (unpaired electron) at the active site of RnR can be destroyed (paired-up) by a stream of electrons. Thus, gentle electrotherapy would disable RnR and interrupt cancer growth [79]. This concept is strongly supported by the results of several animal studies on cancer electrotherapy reported over the years [80–82]. The most remarkable of these was the 1985 study which showed up to 98% reduction in tumor mass—a virtual cure—after only 5 h of gentle electrotherapy over 5 days [82].

Lately, evidence is accumulating of the effectiveness of this therapy on human patients as well [83, 84]. In the case of both animals and humans, there is indisputable experimental evidence that this treatment selectively targets malignant cells since the normal growth of intervening tissues—such as hair follicles—remain unaffected. This is almost certainly due to the fact that the active site of RnR is well-shielded in healthy cells [61], whereas that is not likely to be the case when RnR is in overdrive or frenzied in cancerous tissues.

**CONCLUSIONS**

Using the somatic mutation theory (SMT), which posits that cancer is caused by genetic mutations or “oncogenes,” researchers have been unable to determine the immediate cause of unrestrained cell proliferation, or suggest a pathway for the initiation of the disease by any carcinogen. By focusing on ribonucleotide reductase (RnR)—a pivotal enzyme in DNA synthesis and in cancer growth—these and other aspects of the disease are demystified. For example, this viewpoint reveals the proximate cause of uncontrolled cell growth: the hyper-activity of RnR, as confirmed by studies of retinoblastoma and HPV-related cervical
cancers. This perspective also suggests a way to defeat cancer, as the free radical—essential for the activity of RnR—can be quenched by a stream of electrons.

ACKNOWLEDGEMENTS

Funding. No funding or sponsorship was received for this study or publication of this article. The Rapid Service Fee was funded by the author.

Author Contributions. Jay Kulsh is the sole author on this publication and takes responsibility for the conception and integrity of the work as a whole, and have given their approval for this version to be published.

Disclosures. The author, Jay Kulsh, declares that there are no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. He has nothing to disclose.

Compliance with Ethics Guidelines. This article is based on previously conducted studies and does not contain any new studies with human participants or animals performed by the author.

Data Availability. Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Open Access. This article is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License, which permits any non-commercial use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc/4.0/.

REFERENCES

1. Pollack A: huge genome project is proposed to fight cancer, NYTimes. March 28, 2005(A), p. 1. https://www.nytimes.com/2005/03/28/health/huge-genome-project-is-proposed-to-fight-cancer.html?searchResultPosition=2. Accessed 21 Nov 2022.

2. Cancer World 2013, Dr. James Watson https://archive.cancerworld.net/cover-story/jim-watson-dna-revealed-the-causes-it-may-never-reveal-a-cure/. Accessed 2 Feb 2023.

3. Sung H, Ferlay J, Siegel RL, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209–49. https://doi.org/10.3322/caac.21660.

4. Beardsley T. A war not won. Sci Am. 1994;270(1):130–8. https://doi.org/10.1038/scientificamerican0194-130.

5. Brucher BLD, Jamall IS. Somatic mutation theory—why it’s wrong for most cancers. Cell Physiol Biochem. 2016;38:1663–80. https://doi.org/10.1159/000443106.

6. Joyner MJ, Paneth N, Ioannidis JP. What happens when underperforming big ideas in research become entrenched? J Am Med Assoc. 2016;316(13):1355–6. https://doi.org/10.1001/jama.2016.11076.

7. Sonnenschein C, Soto AM. Over a century of cancer research: Inconvenient truths and promising leads. PLoS Biol. 2020;18(4):e3000670. https://doi.org/10.1371/journal.pbio.3000670.

8. Information taken from the website of American Cancer Society https://cancer.org. Accessed 21 Nov 2022.

9. Anand P, Kunnumakkara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS, Sung B, Aggarwal BB. Cancer is a preventable disease that requires major lifestyle changes. Pharm Res.
10. Shiovitz S, Korde LA. Genetics of breast cancer: a topic in evolution. Ann Oncol. 2015;26(7):1291–9. https://doi.org/10.1093/annonc/mdv022.

11. Garte S. Chapter 5. Individual susceptibility and gene–environment interaction. In: Wild C, Vineis P, Garte S, editors. Molecular epidemiology of chronic diseases. Wiley; 2008. p. 55–69. https://doi.org/10.1002/9780470725726.ch5. (Print ISBN: 9780470027431).

12. Sasiadek M, Karpinski P. Genetic theory of cancer. Short review. Pol J Surg. 2009;81(10):478–85.

13. Raunio H, Kuusisto M, Juvonen RO, Pentikainen OT. Modeling of interactions between xenobiotics and cytochrome P450 (CYP) enzymes. Front Pharmacol. 2015;6:123. https://doi.org/10.3389/fphar.2015.00123.

14. Toyokuni S. Mechanisms of asbestos-induced carcinogenesis. Nagoya J Med Sci. 2009;71(1–2):1–10.

15. D’Orazio J, Jarrett S, Amaro-Ortiz A, Scott T. UV radiation and the skin. Int J Mol Sci. 2013;14(6):12222–48. https://doi.org/10.3390/ijms140612222.

16. Borrego-Soto G, Ortiz-López R, Rojas-Martinez A. Ionizing radiation-induced DNA injury and damage detection in patients with breast cancer. Genet Mol Biol. 2015;38(4):420–32. https://doi.org/10.1590/S1415-4757201500019.

17. Tsutsumi M, Kowa-Sugiyama H, Bolor H, Kogo H, Inagaki H, Yamada K, Taniguchi-Ikeda M, Toda T, Kurahashi H. Screening of genes involved in chromosome segregation during meiosis I: in vitro gene transfer to mouse fetal oocytes. J Hum Genet. 2012;57(8):515–22. https://doi.org/10.1038/jhg.2012.61.

18. Kittler R, Pelletier L, Heninger AK, Slabicki M, Theis M, Miroslaw L, Poser I, Lawo S, Grabner H, Kozak K, et al. Genome-scale RNAi profiling of cell division in human tissue culture cells. Nat Cell Biol. 2007;9(12):1401–12. https://doi.org/10.1038/nclb1659.

19. Wood RD, Mitchell M, Lindahl T. Human DNA repair genes. Mutat Res. 2005;577(1–2):275–83. https://doi.org/10.1016/j.mrfmmm.2005.03.007.

20. Holleman A, den Boer ML, de Menezes RX, Cheok MH, Cheng C, Kazemier KM, Janka-Schaub GE, Gobel U, Graubner UB, Evans WE, et al. The expression of 70 apoptosis genes in relation to lineage, genetic subtype, cellular drug resistance, and outcome in childhood acute lymphoblastic leukemia. Blood. 2006;107(2):769–76. https://doi.org/10.1182/blood-2005-07-2930.

21. Burgio E, Migliore L. Towards a systemic paradigm in carcinogenesis: linking epigenetics and genetics. Mol Biol Rep. 2015;42(4):777–90. https://doi.org/10.1007/s11033-014-3804-3.

22. Tamasi V, Monostory K, Prough RA, Falus A. Role of xenobiotic metabolism in cancer: involvement of transcriptional and miRNA regulation of P450s. Cell Mol Life Sci. 2011;68(7):1131–46. https://doi.org/10.1007/s00018-010-0600-7.

23. Peltomäki P. Lynch syndrome genes. Fam Cancer. 2005;4(3):227–32. https://doi.org/10.1007/s10689-004-7993-0.

24. Davidoff AM. Pediatric oncology. Semin Pediatr Surg. 2010;19(3):225–33. https://doi.org/10.1053/j.sempt Surg.2010.03.007.

25. Information taken from cancer.net—website of American Society of Clinical Oncology, and cancer.org - website of American Cancer Society. Accessed 21 Nov 2022.

26. Knudson A. Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci USA. 1971;68:820–3. https://doi.org/10.1073/pnas.68.4.820.

27. Information obtained from the website of Stanford Medical Center. https://stanfordhealthcare.org/medical-conditions/cancer/multiple-endocrine-neoplasia.html. Accessed 21 Nov 2022.

28. Miklos GLG. The human cancer genome project—one more misstep in the war on cancer. Nat Biotechnol. 2005;23(5):535–7. https://doi.org/10.1038/nbt0505-535.

29. Masters JRW, Lakhani SR. How diagnosis with microarrays can help cancer patients. Nature. 2000;404(6781):921. https://doi.org/10.1038/35010139.

30. Salk JJ, Fox EJ, Loeb LA. Mutational heterogeneity in human cancers: origin and consequences. Annu Rev Pathol. 2010;5:51–75. https://doi.org/10.1146/annurev-pathol-121808-102113.

31. Wu JM, Fackler MJ, Halushka MK, Molavi DW, Taylor ME, Teo WW, Griffin C, Fetting J, Davidson NE, De Marzo AM, et al. Heterogeneity of breast cancer metastases: comparison of therapeutic target expression and promoter methylation between primary tumors and their multifocal metastases. Clin Cancer Res. 2008;14(7):1938–46. https://doi.org/10.1158/1078-0432.ccr-07-4082.

32. Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, Herman P, Kaye FJ, Lindeman N, Boggon

△ Adis
33. Weinberg RA. Coming full circle—from endless complexity to simplicity and back again. Cell. 2014;157(1):267–71. https://doi.org/10.1016/j.cell.2014.03.004.

34. Begley CG, Ellis LM. Drug development: raise standards for preclinical cancer research. Nature. 2012;483(7391):531–3. https://doi.org/10.1038/483531a.

35. DePinho RA. The age of cancer. Nature. 2000;408(6809):248–54. https://doi.org/10.1038/35041694.

36. Tomasetti C, Vogelstein B. Variation in cancer risk among tissues can be explained by the number of stem cell divisions. Science. 2015;347(6217):78–81. https://doi.org/10.1126/science.1260825.

37. Hoover RN. Cancer—nature, nurture, or both. N Engl J Med. 2000;343(2):135–6. https://doi.org/10.1056/NEJM200007133430201.

38. Pritchard-Jones K, Kaatsch P, Steliarova-Foucher E, Stiller CA, Coebergh JW. Cancer in children and adolescents in Europe: developments over 20 years and future challenges. Eur J Cancer. 2006;42(13):2183–90. https://doi.org/10.1016/j.ejca.2006.06.006.

39. Bleyer A, O'Leary M, Barr R, Ries LAG. (eds): Cancer epidemiology in older adolescents and young adults 15–29 years of age, including SEER incidence and survival: 1975–2000. NIH 2006, Pub. No. 06-5767. National Cancer Institute, Bethesda (MD). https://seer.cancer.gov/archive/publications/aya/aya_mono_complete.pdf. Accessed 2 Feb 2023.

40. Li N, Zhai Z, Zheng Y, Lin S, Deng Y, Xiang G, Yao J, Xiang D, Wang S, Yang P, et al. Association of 13 occupational carcinogens in patients with cancer, individually and collectively, 1990–2017. JAMA Netw Open. 2021;4(2):e2037530. https://doi.org/10.1001/jamanetworkopen.2020.37530.

41. Clarkson B, Boyse EA. Possible explanation of the high concordance for acute leukemia in monozygotic twins. Lancet. 1971;7701:699–701.

42. Mack TM, Cozen W, Shibata DK, Weiss LM, Nathwani BN, Hernandez AM, Taylor CR, Hamilton AS, Deapen DM, Rappaport EB. Concordance for Hodgkin's disease in identical twins suggesting genetic susceptibility to the young-adult form of the disease. N Engl J Med. 1995;332(7):413–9. https://doi.org/10.1056/NEJM199502163320701.

43. Lichtenstein P, Holm NV, Verkasalo PK, Iliadou A, Kaprio J, Koskenvuo M, Pukkala E, Skytthe A, Hemminki K. Environmental and heritable factors in the causation of cancer—analyses of cohorts of twins from Sweden, Denmark, and Finland. N Engl J Med. 2000;343(2):78–85. https://doi.org/10.1056/nejm2000071313340201.

44. Mucci LA, Hjelmborg JB, Harris JR, Czene K, Havelick DJ, Scheike T, Graff RE, Holst K, Moller S, Unger RH, et al. Familial risk and heritability of cancer among twins in Nordic countries. J Am Med Assoc. 2016;315(1):68–76. https://doi.org/10.1001/jama.2015.17703.

45. Nogrady B. How cancer genomics is transforming diagnosis and treatment. Nature. 2020;579(7800):S10–1. https://doi.org/10.1038/d41586-020-00845-4.

46. Nicholson JM. Will we cure cancer by sequencing thousands of genomes? Mol Cytogenet. 2013;6:57. https://doi.org/10.1186/1755-8166-6-57.

47. Heng HH. The genomic landscape of cancers. In: Ujvari B, Roche B, Thomas F, editors. Ecology and evolution of cancer. Elsevier; 2017. p. 69–86. https://doi.org/10.1016/B978-0-12-804310-3.00005-3.

48. Brock A, Huang S. Precision oncology: between vaguely right and precisely wrong. Cancer Res. 2017;77(23):6473–9. https://doi.org/10.1158/0008-5472.CAN-17-0448.

49. Sonnenschein C, Soto AM. The somatic mutation theory of carcinogenesis: why it should be dropped and replaced. Mol Carcinogen. 2000;29:1–7. https://doi.org/10.1002/(SICI)1098-2744(200012)29:4%3C9::AID-MC100%3E3.0.CO;2-W.

50. Kato S, Lippman SM, Flaherty KT, Kurzrock R. The conundrum of genetic “drivers” in benign conditions. J Natl Cancer Inst. 2016. https://doi.org/10.1093/jnci/djw036.

51. Heng HH. Chapter 1. From Mendelian genetics to 4D genomics. Genome chaos. Academic Press; 2019. p. 1–52. https://doi.org/10.1016/B978-0-12-813635-5.00001-X.

52. Mintz B, Illmensee K. Normal genetically mosaic mice produced from malignant teratocarcinoma cells. Proc Natl Acad Sci USA. 1975;72(9):3585–9. https://doi.org/10.1073/pnas.72.9.3585.

53. Israel BA, Schaeffer WI. Cytoplasmic suppression of malignancy. In: Vitro Cell Dev Biol. 1987;23(9):627–32. https://doi.org/10.1007/BF02621071.
54. Israel BA, Schaeffer WI. Cytoplasmic mediation of malignancy. In Vitro Cell Dev Biol. 1988;24(5):487–90. https://doi.org/10.1007/BF02628504.

55. Li L, Connelly MC, Wetmore C, Curran T, Morgan JL. Mouse embryos cloned from brain tumors. Cancer Res. 2003;63(11):2733–6.

56. Seyfried TN. Cancer as a mitochondrial metabolic disease. Front Cell Dev Biol. 2015;3:43. https://doi.org/10.3389/fcell.2015.00043.

57. Apple S: An old idea, revived: starve cancer to death. New York Times. Page 64 of the Sunday Magazine. May 12 2016. https://www.nytimes.com/2016/05/15/magazine/warburg-effect-an-old-idea-revived-starve-cancer-to-death.html. Accessed 21 Nov 2022.

58. Farber S, Diamond LK, Mercer RD, Sylvester Jr RF, Wolff JA. Temporary remissions in acute leukemia in children produced by folic acid antagonist, 4-Aminopteroyl-glutamic acid. N Engl J Med. 1948;238(23):787–93. https://doi.org/10.1056/nejm194806032382301.

59. Nordlund P, Sjöberg B-M, Eklund H. Three-dimensional structure of the free radical protein of ribonucleotide reductase. Nature. 1990;345(6276):593–8. https://doi.org/10.1038/345593a0.

60. Weber G. Biochemical strategy of cancer cells and the design of chemotherapy: G. H. A. Clowes memorial lecture. Cancer Res. 1983;43(8):3466–92.

61. Nordlund P, Sjöberg B-M, Eklund H. Three-dimensional structure of the free radical protein of ribonucleotide reductase. Nature. 1990;345(6276):593–8. https://doi.org/10.1038/345593a0.

62. Aye Y, Li M, Long MJ, Weiss RS. Ribonucleotide reductase and cancer: biological mechanisms and targeted therapies. Oncogene. 2015;34(16):2011–21. https://doi.org/10.1038/onc.2014.155.

63. Knighton LE, Delgado LE, Truman AW. Novel insights into molecular chaperone regulation of ribonucleotide reductase. Curr Genet. 2019;65(2):477–82. https://doi.org/10.1007/s00294-018-0916-7.

64. Pontarini G, Fijolek A, Pizzo P, Ferraro P, Rampazzo C, Pozzan T, Thelander L, Reichard PA, Bianchi V. Ribonucleotide reduction is a cytosolic process in mammalian cells independently of DNA damage. Proc Natl Acad Sci USA. 2008;105(46):17801–6. https://doi.org/10.1073/pnas.0808198105.

65. Strand KR, Karlsen S, Kolberg M, Rohr AK, Gorbitch CH, Andersson KK. Crystal structural studies of changes in the native dinuclear iron center of ribonucleotide reductase protein R2 from mouse. J Biol Chem. 2004;279(45):46794–801. https://doi.org/10.1074/jbc.M407346200.

66. Liu A, Potsch S, Davydov A, Barra AL, Rubin H, Graslund A. The tyrosyl free radical of recombinant ribonucleotide reductase from mycobacterium tuberculosis is located in a rigid hydrophobic pocket. Biochemistry. 1998;37(46):16369–77. https://doi.org/10.1021/bi981471p.

67. Zeljezic D. Assessment of potential carcinogenicity by quantitative structure-activity relationship (QSAR). In: Larramendy ML, Soloneski S, editors. Genotoxicity—a predictable risk to our actual world. IntechOpen; 2018. p. 61–80. https://doi.org/10.5772/intechopen.75420.

68. Wang N, Zhan T, Ke T, Huang X, Ke D, Wang Q, Li H. Increased expression of RRM2 by human papillomavirus E7 oncoprotein promotes angiogenesis in cervical cancer. Br J Cancer. 2014;110:1034–44. https://doi.org/10.1038/bjc.2013.817.

69. Su YF, Wu TF, Ko JL, Tsai HT, Tee YT, Chien MH, Chou CH, Lin WL, Low HY, Chou MY, et al. The expression of ribonucleotide reductase M2 in the carcinogenesis of uterine cervix and its relationship with clinicopathological characteristics and prognosis of cancer patients. PLoS One. 2014;9(3):91644. https://doi.org/10.1371/journal.pone.0091644.

70. Huh KW, DeMasi J, Ogawa H, Nakatani Y, Howley PM, Munger K. Association of the human papillomavirus type 16 E7 oncoprotein with the 600-kDa retinoblastoma protein-associated factor, p600. Proc Natl Acad Sci USA. 2005;102:11492–7. https://doi.org/10.1073/pnas.0505337102.

71. Stubbe J, Nocera DG. Radicals in biology: your life is in their hands. J Am Chem Soc. 2021;143:13463–72. https://doi.org/10.1038/onsc.2014.155.

72. Shao J, Liu X, Zhu L, Yen Y. Targeting ribonucleotide reductase for cancer therapy. Expert Opin Ther Targets. 2013;17:1423–37. https://doi.org/10.1517/14728222.2013.840293.

73. Hanahan D. Hallmarks of cancer: new dimensions. Cancer Discov. 2022;12:31–46. https://doi.org/10.1158/2159-8290.CD-21-1059.

74. Misko TA, Liu YT, Harris ME, Oleinick NL, Pink J, Lee HY, Dealwis CG. Structure-guided design of anti-cancer ribonucleotide reductase inhibitors. J Enzyme Inhib Med Chem. 2019;34(1):438–50. https://doi.org/10.1080/14756366.2018.1545226.

75. Agrawal KC, Booth BA, Sartorelli AC. Potential antitumor agents. 7. 4’-diethoxybenzene derivatives of -(N)-heterocyclic carboxaldehyde
76. Greene BL, Kang G, Cui C, Bennati M, Nocera DG, Drennan CL, Stubbe J. Ribonucleotide reductases: structure, chemistry, and metabolism suggest new therapeutic targets. Annu Rev Biochem. 2020;89:45–75. https://doi.org/10.1146/annurev-biochem-013118-111843.

77. Zhou B, Su L, Hu S, Hu W, Yip ML, Wu J, Gaur S, Smith DL, Yuan YC, Synold TW, et al. A small-molecule blocking ribonucleotide reductase holoenzyme formation inhibits cancer cell growth and overcomes drug resistance. Cancer Res. 2013;73:6484–93. https://doi.org/10.1158/0008-5472.CAN-13-1094.

78. Wang R, Xu Z, Tian J, Liu Q, Dong J, Guo L, Hai B, Liu X, Yao H, Chen Z, et al. Pterostilbene inhibits hepatocellular carcinoma proliferation and HBV replication by targeting ribonucleotide reductase M2 protein. Am J Cancer Res. 2021;11:2975–89.

79. Kulsh J. Targeting a key enzyme in cell growth: a novel therapy for cancer. Med Hypotheses. 1997;49:297–300. https://doi.org/10.1016/S0306-9877(97)90193-6.

80. Humphrey CE, Seal EH. Biophysical approach toward tumor regression in mice. Science. 1959;130:388–90. https://doi.org/10.1126/science.130.3372.388.

81. Schauble MK, Habal MB, Gullick HD. Inhibition of experimental tumor growth in hamsters by small direct currents. Arch Pathol Lab Med. 1977;101(6):294–7.

82. David SL, Absolom DR, Smith CR, Gams J, Herbert MA. Effect of low level direct current on in vivo tumor growth in hamsters. Cancer Res. 1985;45:5625–31.

83. Oji C, Ani J. Destruction of an advanced malignant tumour by direct electrical current -case report. Health. 2010;2(9):1049–53. https://doi.org/10.4236/health.2010.29154.

84. Kulsh J. Low-level electric current and cancer—a promising, but languishing non-toxic cancer therapy. Explore J Sci Health. 2014;10(1):53–4. https://doi.org/10.1016/j.explore.2013.10.004.