Destabilization of the DNA Double Helix in Cancer
Mirko Beljanski's Theory of Carcinogenesis and Anti-Cancer Extracts

by John Hall, PhD

Two major discoveries dating from the middle of the 20th century fueled the growth of molecular biology and shaped our ideas about the critical role of DNA in the onset of cancer. At Rockefeller University in New York (1944) Avery, McCarty, and MacLeod identified DNA, a complex of four building blocks called nucleotides, as the genetic material.[1] At the Medical Research Council in Cambridge (1953) Watson and Crick reported that multiples of these four nucleotides are strung together in long complementary polymers that wrap around each other forming a double helix.[2] The concept that DNA contains genetic information – specific sequences of nucleotides that code for specific proteins – has had a far greater impact on cancer research than the idea that DNA exists as a double helical structure. The mainstream theories of carcinogenesis are information-based and contend that the coding regions of a special set of genes are corrupted by mutations, resulting in altered function of the corresponding proteins, and leading to aggressive cell division and ultimately to cancer.[3] Indeed, this view has been vindicated to the extent that mutations in some classes of genes, notably oncogenes and tumor suppressors, have been correlated with various types of cancer.[4]

In contrast to this information-based model, a physical model suggesting that disruption in the structure of the DNA double helix is a central factor in carcinogenesis might seem at the very least unlikely, if not simply off the mark. Yet there is growing evidence that a unified view of cancer, one that will support more successful diagnostics and therapies, must incorporate this physical model, which isn't new, but is certainly ripe for rediscovery and reappraisal.[5-8]

Starting at the Pasteur Institute in Paris (1951) the biochemist Mirko Beljanski was well-positioned to assimilate the discoveries concerning the genetic and physical properties of DNA and to develop his own theory of carcinogenesis. He accomplished this in a series of innovative experiments that can be summarized in three stages.

1. DNA Structure is Destabilized in Cancer Cells

Beljanski's first goal was to examine the difference between DNAs isolated and purified from normal and cancerous cells. What is remarkable is that he addressed this question at the level of the physical structure of the DNA rather than at the level of the genetic content of the DNA. The identity of the nucleotides at successive positions along the DNA polymer is known as a DNA sequence. Obtaining this information was the central goal of the human genome project and finding the critical differences between the DNA sequences of normal and malignant cells has been a central focus of cancer research. In complete contrast to this approach, Beljanski sought to identify the differences between normal and cancer DNAs at the level of the physical structure of the DNA duplex by testing for changes in the stability of the double helix.

Beljanski used two assays to show that DNA from cancer cells is different from the DNA of normal cells.[9] The first assay measured the difference in absorption of ultraviolet light by the DNA from the two sources. The absorption of light at specific wavelengths is a characteristic of many biomolecules. In this case, Beljanski used UV absorption as a measure of how tightly the two strands of DNA are held together in the double helix; the greater the strand separation the higher the absorption of UV. As controls for the experiment he confirmed that the absorption of samples in which the two strands are completely separated by chemical treatment is significantly higher than the absorption of DNA from healthy tissues (~40%). The absorption of DNA from cancer cells was consistently higher (by 1-5%) than the results found for normal DNAs. Beljanski concluded that the chemical bonds that hold the double helix together are reproducibly disrupted in cancer DNA which results in more openings or loops than are present in normal DNA. He referred to this pattern of relaxation in the DNA of cancer cells as destabilization.

Beljanski was well aware that the opening and closing of the DNA helix in cells is part of the dynamic of life: whenever the DNA replicates or the information it contains is expressed, the strands must be separated to enable access of the enzymatic machinery that perform duplication and transcription. In a healthy cell the opening of the DNA is well regulated and the loops are re-closed when these processes are complete. The persistent openings Beljanski found in cancer DNA could be the cause of the deregulation of DNA metabolism that is the hallmark of most cancers: destabilization of the DNA structure could be associated with excess replication and aberrant gene expression. This suggested a second assay. If the DNA is destabilized into frequent loops then it should serve as a more active template for enzymatic reactions performed in the test tube than the more tightly wound duplexes characteristic of purified normal.[9]

This prediction was confirmed in experiments demonstrating that cancer DNA replicated more rapidly than normal DNA as measured by the incorporation of specially labeled nucleotides into polymeric form. Given the exact same amount of DNA template, nucleotide building blocks, and the enzyme known as DNA polymerase, the reaction containing cancer DNA consistently synthesized more new DNA. Thus DNA destabilization was correlated with enhanced DNA synthesis.[5]

2. The Oncotest: Carcinogens as DNA Destabilizers

Beljanski's next step was to conceive of the differential structure and template activity of normal versus cancer DNA as an assay in its own right for detecting whether a given substance, naturally occurring or man made, would have an effect on DNA stability. In this case, UV absorption and the rate of DNA replication serve as the indicators of a compound's effect.

Beljanski called his assay the Oncotest and it stands as an elegant, though under-appreciated, development in the history of cancer research. Beljanski took his observations seriously: the relatively open structure of cancer DNA was perceived as the central feature of cancer cells and so the process of destabilization was cast as the central event in carcinogenesis. The question was: what substances that we are exposed to cause this to happen? He had already designed two assays that measured the extent of instability, what remained was the addition of compounds to these assays to test their effect on the structure of the DNA; compounds that enhanced the UV absorption or the level of *in vitro* DNA synthesis should be considered to have carcinogenic properties.[6]

At first, Beljanski introduced known carcinogens (e.g. benzene, nicotine) into the Oncotest and discovered that these compounds enhanced UV absorption and DNA synthesis when the DNA template was normal. When the DNA in the test was isolated from cancer cells he found that it was more sensitive to the effects of the carcinogen showing significantly higher levels of UV absorption and DNA synthesis than normal DNA templates. Beljanski speculated that the already open structure of the cancer DNA is what makes it more susceptible to the effect of carcinogens, an effect that appeared to consist of promoting even more openings in the DNA structure.

Further experiments with the Oncotest using a wider range of compounds provided three significant results. First, Beljanski found that steroid hormones showed carcinogenic potential in the test. This result was unexpected because hormones are biochemical regulators that occur naturally in the body. Why would testosterone and progesterone, for example, behave like carcinogens in the Oncotest? Biomedical research has been coming to grips with this issue and we know, for example, that hormone replacement therapies for women may lead to a higher incidence of breast cancer.[10]

In addition to finding many substances such as lactose, saccharin, and cholesterol that were neutral in the Oncotest, Beljanski also identified a class of compounds that were carcinogenic in the Oncotest, but which did not show mutagenic potential in the mainstream Ames test used to classify cancer causing compounds.[11] This result illustrates again Beljanski's departure from the information-based model of carcinogenesis. The Oncotest revealed that a compound could show carcinogenic properties (by affecting DNA structure) without necessarily being a mutagen, which are widely understood to cause cancer by altering the sequence of the DNA.

Finally, Beljanski tested several compounds used for anti-cancer chemotherapy and found many of them to be carcinogens. This proved to be consistent with other results and it is now common knowledge that many chemotherapeutic drugs are themselves potent carcinogens.

3. What is the Opposite of a Carcinogen?

The Oncotest is a powerful tool for evaluating potential carcinogens and the fact that it is not more widely adopted is a subject for another article. Beljanski himself transcended the application of the Oncotest as a screen for carcinogens by posing a fundamental question: why not use the test to screen for compounds that have the opposite of the carcinogenic effect? Why not run the test with cancer DNA templates and look for compounds that reduce instead of increase UV absorption and DNA synthesis?[12] Beljanski focused on screening natural compounds, not just because of their diversity, but because he reasoned that natural compounds would be more amenable to use as anti-cancer agents in humans. Following analysis of several hundred compounds Beljanski identified a small number that reduced

Oncotest Assay

UV absorption and template activity of cancer DNA in the Oncotest. What is more, these extracts did not affect the results obtained with normal DNA. These were the extracts Beljanski was looking for. Two of the extracts he found, derived from tropical plants (*Pao Pereira* and *Rauwolfia Vomitoria* which he named PB100 and BG8 respectively), were particularly effective and became the focus of his research.[13]

What had Beljanski accomplished? Like any research scientist who focuses on cancer he looked for a fundamental difference between cancer cells and normal cells. He identified this difference as an alteration in the structure of cancer DNA – not at the level of mutations in the DNA sequence – but in disruptions of the DNA double helix. He devised a simple laboratory test for identifying compounds that could cause and promote the destabilization of DNA and showed that known carcinogens acted in exactly this way. He then went on use this test to search for compounds with effects that were the opposite of carcinogens. In his work with these compounds Beljanski created a novel frontier for cancer therapy.

Beljanski's Extracts: Selective Anti-Cancer Effects

The Beljanski extracts were subjected to a long series of tests to examine their effect on cultured cancer cells, on animals with various kinds of cancer, and ultimately in numerous human case studies.[14-18] The extracts showed several consistent and remarkable properties. First, they stopped the proliferation of cancer cell lines maintained in the laboratory, while sparing healthy cells. They were toxic to cancer cells in mice, but did no harm to healthy mice. They have proven to have anti-cancer effects on a range of human malignancies, but have shown no significant side effects. In a word, the activity of these extracts is selective to cancer DNA, to cancer cells, and to organisms with cancer. They are currently sold as nutritional supplements by Natural Source International (a company led by

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Beljanski's daughter and son-in-law) under the commercial names Pao V®, Pao V FM®, and Rovol V®.

How do they work? Beljanski isolated what he thought to be the active molecules from the extracts and found that they were structurally related alkaloids of the beta-carboline class. Several studies have shown that these molecules bind to DNA and Beljanski reasoned that their activity in cancer cells was predicted by their activity in the Oncotest: they inhibit synthesis of cancer DNA templates. In cancer cells they apparently act by entering the already open strands of the DNA where they intercalate between adjacent nucleotides, interfere with DNA replication, and thereby induce cell death. Beljanski also argued that alteration of the electrical properties of the membranes of cancer cells specifically enhances the penetration of the compounds into these cells.[18]

Beljanski's Extracts: Synergy with Conventional Cancer Therapies

In several animal studies, Beljanski tested the anti-cancer effects of his extracts when combined with various drugs used for chemotherapy. Mice injected with YC8 cancer cells normally succumb to cancer and die within four weeks. When these animals were treated with either the anti-cancer drug daunorubicin or with the BG8 extract, 30-40% of the animals survived past ninety days. However, when treated with a combination of daunorubicin and BG8 over 90% of the mice lived beyond ninety days. These data suggested that the extracts might be effective in combination therapies including anti-mitotic drugs.

Although the Beljanski extracts are new to the US, the products have been used in Europe for some time where numerous case studies, testimonials, and clinical histories provide additional information concerning their utility in humans (See the websites listed below for more information). The extracts have frequently been used in conjunction with mainstream cancer treatments. Indeed, they have proven to be synergistic with low to moderate doses of chemotherapy and radiation therapy. In these cases, they appear to support the benefits of the conventional therapies while alleviating at least some of the well-known side effects.[20]

The two extracts are often used together and seem to be synergistic with each other, but differences in the chemical structure of their active compounds has led to somewhat different uses. For example, the compound in Pao V®, flavopereirine, is smaller than the compound in Rovol V® (alstonine) and can be used for brain tumors because it crosses the blood-brain barrier. In contrast, Rovol V® seems to be more effective than Pao V® in hormonally-related tumors such as prostate and breast.

Beljanski Extracts: A Role in Cancer Prevention?

Finally, several factors suggest that these extracts are prime candidates for cancer prevention. First and foremost, they do not cause side effects, which is paramount for any preventive agent. Second, it is possible that the goal of prevention, which is to knock off a small population of cancer cells before a tumor can develop, could be attained by modest doses of these extracts. One can foresee a combination of the two extracts being taken periodically by individuals at high risk or even by healthy middle-aged men and women with the aim of preventing prostate or breast cancer. And although many questions remain regarding the optimal use and the mechanism of action of Pao® and Rovol®, efforts to formalize their application and to improve our understanding of their anti-cancer effect are underway. For example, a major clinical study to assess the activity of these extracts in the prevention of prostate cancer is due to begin later this year.

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For Further Information on Natural Source Products and Dr. Beljanski's scientific research please visit the following websites:
www.natural-source.com
www.beljanski.com
www.mbschachter.com
http://www.findarticles.com/cf_dls/m0ISW/244/111271889/p1/article.jhtml

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