

Profiles and Legacies

Natural Products: Nature's Gift

Molecules for Cancer Prevention and Treatment

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Original manuscript submitted: 09/05/07
Manuscript accepted: 09/05/07

This manuscript has been published online, prior to printing for *Cancer Biology & Therapy*, Volume 6, Issue 9. Definitive page numbers have not been assigned. The current citation is: *Cancer Biol Ther* 2007; 6(9):

<http://www.landesbioscience.com/journals/cbt/article/4987>

Once the issue is complete and page numbers have been assigned, the citation will change accordingly.

KEY WORDS

cancer chemoprevention, natural products, signal transduction, apoptosis induction, environmental carcinogens, cancer treatment, tumor initiation, tea polyphenols, preclinical trials, custom tailoring, combination

ABSTRACT

When the editor of *Cancer Biology and Therapy* called me about writing a contribution to a series called Profiles and Legacies, two thoughts came to my mind. The first was that he dialed a wrong number. The second was that maybe he could not find someone with a "name and fame" to write it. In response to my first thought, he convinced me that he was calling the right person. Thus, I researched the past writers of the Profiles and Legacies section of the journal, and was pleased to note that eminent scientists have been profiled in this section of the journal. Though I am still uncertain why I am invited, I am pleased to be in the company of such an eminent group of scholars, and I fully realize that I have a big challenge before me.

HOW I BECAME WHAT I AM

In 1971, at 24 years of age, I obtained my Ph.D. degree in Biochemistry with a thesis titled, *Chemical Studies on Drug Metabolism in Relation to Enzyme Induction.* At that time I had no knowledge of cancer etiology, as my knowledge-base was limited to cytochrome P450 and related xenobiotic metabolism aspects. I came to the United States in 1974 to obtain postdoctoral training under the late Professor Edward Bresnick, at the Medical College of Georgia. I remember Ed taught me aryl hydrocarbon hydroxylase assay and explained to me how it is the key enzyme for an inert ubiquitous environmental agent, benzo(a)pyrene, to become a carcinogen that then binds to DNA leading to cancer initiation. He left me alone on the bench to work with this enzyme, but notoriously I began working on the other side of the coin, on detoxification enzymes. I then moved to the National Institute of Environmental Health Sciences, where I became a pseudo-expert on detoxification enzymes while most other people there were concentrating on how environmental agents become carcinogens.

When I became a faculty member at Case Western Reserve University, I decided that I would combine the expertise of metabolic activation and detoxification, which at the time was considered a key way to regulate carcinogenesis. In the early 1980s, this was the main frame of cancer chemoprevention research. I have been studying cancer chemoprevention since then. I became fascinated with the idea of diet and cancer, which led me to focus on dietary substances for cancer chemoprevention. In this area of science, I was inspired by the work of Drs. Allan Conney, Paul Talalay and Lee Wattenberg, to name a few. Over the years, chemoprevention research has dramatically changed for good and bad. So far, I have spent over 25 years in establishing many chemopreventive agents and establishing molecular targets for cancer chemoprevention using skin, prostate and lung cancer models. I was always fortunate to have a highly dedicated team of extremely talented postdoctoral fellows in my laboratory to conduct the work for which I shared the credit. I will refrain from naming them here because the list is very long—as is evident from the fact that, at this time, there are a large number of my trainees who are running their own very successful research programs in many universities around the nation, conducting possibly more advanced science than I am capable of conceiving and executing.

NATURAL PRODUCTS FOR CANCER

I will share my thoughts about the usefulness of natural products, derived from plant sources, for preventing and treating cancer. Through the research efforts of the last fifty years, undeniable, significant progress has been made in: (I) understanding processes

ABOUT DR. MUKHTAR

Dr. Hasan Mukhtar, Ph.D., is a Helfaer Professor of Cancer Research, Director and Vice Chair for Research, Department of Dermatology, School of Medicine and Public Health, University of Wisconsin, Madison, Wisconsin USA. He is also the Co-leader of Cancer Chemoprevention Program, Paul P Carbone Comprehensive Cancer Center, University of Wisconsin. Dr. Hasan Mukhtar was born on January 1, 1947 in Lucknow, India. After obtaining his Ph.D. from the Kanpur University while working at the Central Drug Research Institute (Lucknow) in 1971, he served at the same Institute for another three years. Thereafter, in 1974, he joined Medical College of Georgia, Augusta, as post doctoral fellow under the mentorship of late Professor Edward Bresnick, and in 1976 moved as a Visiting Associate in the Laboratory of Pharmacology at the National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. In 1980, Dr. Mukhtar joined the faculty of the Department of Dermatology, Case Western Reserve University, Cleveland, Ohio, as an Assistant Professor where he rapidly rose to the position of full professor in 1987. At Case Western Reserve University, he also held various academic and administrative appointments including Director, Dermatology Research with joint appointments in Departments of Environmental Health Sciences, Physiology and Biophysics and Radiation Oncology and was the Co-Director of NIAMS funded Skin Diseases Research Center. Then in 2002, he moved to his current position at the University of Wisconsin, School of Medicine and Public Health, Madison. He serves as an Editorial Board member/Associate Editor of 22 scientific journals in diversified fields of cancer, pharmacology, toxicology, biochemistry, dermatology and photobiology that include Clinical Cancer Research, Cancer Research, Life Sciences and Toxicology, and Applied Pharmacology. He serves on grant review committees of National Institutes of Health, American Cancer Society, Department of Veterans Affairs, Department of Defense and many private organizations. Dr Mukhtar has made major contributions in identifying novel diet-based agents and in defining molecular targets for cancer chemoprevention. Dr. Mukhtar is an author of 384 original peer reviewed publications, many in very high impact journals and is an author of 64 book chapters and two books.



the result of multiple rounds of clonal expansion of somatic cells that acquire a selective and uncontrolled growth advantage as a result of acquired mutations in one or more genes that control cellular proliferation and death. Thus, the tumorigenesis process for full-blown malignancy is generally very long, possibly as long as two or three decades. If we can prolong this process, cancer development may be delayed.

Four possible strategies that may control the occurrence of tumor initiation exemplified by DNA damage, and the spread of initiated cells (tumor promotion and progression) to develop cancer, thereby reducing the mortality and morbidity associated with it, include: (I) prevention, (II) early diagnosis and intervention, (III) successful eradication of localized cancer and (IV) improved management of nonlocalized cancer. Among these, prevention of cancer appears to be the most practical approach, because it saves the trouble of being sick and living with the fear associated with its hopeless consequences. This notion is consistent with the history of medicine, which teaches us that greatest gains in health care came from early diagnosis and prevention, and not from treatment. One preventive approach is through chemoprevention which, by definition, is a means of cancer management in which the occurrence of the disease can be entirely prevented, slowed or reversed by the administration of one or more naturally occurring and/or synthetic compounds. The expanded definition of cancer chemoprevention also engulfs the therapy of precancerous lesions. Thus, the practical definition of chemoprevention could be "slowing the journey of normal cells to malignancy." This break in the march could occur at any step of the process. Thus, I will call it a great success if we can slow down the time frame of this journey in a measurable way. The chemopreventive compounds often, though erroneously, are also called anticarcinogens. For human usage, these compounds should, ideally, have the following attributes: (a) little or no adverse effects, (b) high efficacy at multiple sites, (c) effectiveness at dose levels, (d) activity following oral usage, (e) known mechanism of action, (f) low cost, (g) history of usage by the human population and above all, (h) general human

that lead the march of normal cells to malignant lesions, (II) early diagnosis of cancer, (III) surgical removal of malignant lesions and (IV) radiation and other treatment regimens for cancer. All of these have helped in prolonging the life span of many cancer patients. However, although our knowledge of cancer biology has advanced considerably in the last fifty years, neither the incidence of cancer nor the rate of death due to cancer has changed during that time. Most drugs currently in use for treatment of cancer have limitations because they are generally very toxic, highly inefficient in completely and selectively killing cancer cells, and being very expensive are well beyond the economic reach of the general population. Satisfactory treatment of cancer without these disadvantages is a serious need of mankind.

CANCER CHEMOPREVENTION

Extensive research on cancer biology has taught us that tumorigenesis is a multistep process associated with accumulation of genetic alterations in somatic cells. The progression of a normal cell through preneoplasia to frank neoplasia and then invasion and metastasis is

acceptability. It is almost impossible that any synthetic agent could fulfill all criteria. My personal view is that such agents have no future for human cancer management through chemoprevention. Unfortunately, we have spent a lot of time, effort and money in the direction of synthetic agents, to little avail. Consistent with this view, for the past twenty years or so, there has been intense activity in building an armamentarium of chemopreventive substances present, as minor dietary constituents, in a variety of food and beverages consumed by humans. In Table 1, a selected list of those agents is presented. It is impressive that many of these agents, in addition to exhibiting cancer chemopreventive effects are also demonstrating cancer chemotherapeutic effects. Further, some of these agents are capable of further sensitizing known cancer chemotherapeutic effects of anticancer drugs. This offers an exciting possibility that some of these could be used as an adjunct to classical anticancer drugs which could substantially reduce the toxicity associated with them. Nature has gifted them to us, along with many other agents derived from plants, which can modulate the expression of one or more signaling pathways, that play a critical role in cancer development. Abundant preclinical data, in many animal tumor bioassay protocols, shows that these agents can slow the process of carcinogenesis. Our challenge is to take full advantage of this unique resource for human cancer prevention.

A DISCONNECT BETWEEN CANCER CHEMOPREVENTIVE EFFECTS BETWEEN PRECLINICAL STUDIES AND HUMAN CLINICAL TRIALS

Despite very convincing preclinical data in animal models, most clinical trials of agents (either synthetic or diet-based) done to date have not yielded very promising outcomes. The simplest explanation of this disconnect is that either we do not know how to conduct animal studies that could be translated to humans or we do not know how to conduct clinical trials. By thinking deep into the issue of this disconnect, many thoughts come to mind: (I) humans do not eat well-defined, rationed diets as they like to eat food, (II) humans vary in their genetic makeup and (III) humans' occupations, environmental and other factors are also variable. In addition to these apparent issues, there is one more complexity. Most often for obtaining preclinical data we employ artificially high concentrations of carcinogens and devise protocols to mimic chemopreventive regimens. However, when we test these agents in humans, we consider many issues like pharmacokinetics, bioavailability and metabolism of agents being tested. Unfortunately, in general, we do not mimic chemopreventive regimens and rely heavily on surrogate biomarkers. I will cite one example of the disconnect while evaluating chemopreventive effect of green tea polyphenols in animal models of prostate cancer, we let animals drink green tea ad libitum as the sole source of drinking fluid. Using this regimen, remarkable preventive effects have been observed. How can we duplicate this study in humans as they will get bored by drinking green tea and nothing else? Thus, while conducting human trials, 800 mg of green tea polyphenol capsule is given per day and surrogate end point marker of PSA measurement in serum and apoptosis measurements in tissue biopsies at one or two time points is done. The obvious difficulty in conducting chemoprevention studies in humans forces us to develop protocols which are significantly different from preclinical protocols and asks questions which are far beyond those asked in animal studies. One way to handle this very complex issue is to develop human protocols

and first mimic them in preclinical settings. Only when encouraging preclinical data are generated, we execute the human protocol. This would necessitate much close collaboration with animal experimentalists and clinical trials.

CUSTOM TAILORING OF CHEMOPREVENTIVE REGIMEN FOR HUMAN USE

Because cancer arises as a result of several genetic mutations resulting in defects in multiple signaling pathways, it is unlikely that any single agent may prove to be totally effective and beneficial in reducing cancer outcomes on a long-term basis. To achieve greater and long lasting effects, it is my view that naturally occurring agents could be combined in a manner that could be more beneficial and at the same time do not exhibit toxicity. From laboratory studies, we learned that several dietary agents that are effective chemopreventive agents in one experimental setting can enhance or have no effects on carcinogenesis in another experimental setting. Thus, custom tailoring of chemopreventive regimens with known mechanisms targeted to individual need appears more appropriate. This concept of combining chemopreventive agents to achieve greater benefits is being increasingly appreciated and investigators are conducting studies utilizing a combination of natural agents and chemotherapeutic drugs. There is also a need to understand genetic, environmental, and lifestyle factors that influence carcinogenesis in humans, and to use this information to help in the selection of an appropriate cancer chemopreventive regimen in individuals with a high risk for cancer development. This approach could be extremely important when a promising chemopreventive agent demonstrates significant efficacy but may produce toxic effects at higher doses. A combined treatment with drugs having different mechanisms is attractive since they could attack the carcinogenesis pathways at more than one site. This could result in therapy with an additive or a synergistic effect against cancer growth and development.

In the light of the observations suggesting more beneficial effects with a combination of chemopreventive agents at low doses, it is appropriate to design experiments containing a mixture of substances that target multiple signaling pathways. Although, ideally, this may prove to be more advantageous, realistically speaking such approaches may have to be monitored much more carefully for any undesirable toxic effects.

FUTURE PERSPECTIVES

The prevailing wisdom of cancer chemoprevention has been to find effective agents, with acceptable or no toxicity and use them for chemoprevention in relatively healthy people or individuals at high risks for cancer development. This concept has not provided the desired results, possibly because of the facts that one single agent, in spite of being effective in highly optimized laboratory conditions have yielded disappointing results in clinical trials, mainly because human populations with the disease could not be optimized. Humans differ genetically and in lifestyle, dietary habits, environmental exposures among many other factors. Moreover, most of the single agents tested under laboratory conditions have been found to be effective against only one or few targets, making it impossible for a single agent to be effective against all cancers.

My laboratory, through its extensive studies with different dietary agents, has come up with a three-step approach. In the first step of

Table 1 **Natural products with established chemopreventive and/or chemotherapeutic effects.**

Dietary agent	Major dietary source	Cancer target site	Chemopreventive and / or Chemotherapeutic effects	Clinical trials / Case-control studies in humans
EGCG (Polyphenol)	Green tea	Skin, Lung, Prostate, Breast, Liver, Gastrointestinal tract, Pancreas, Bladder and other cancers	Demonstrated	Demonstrated
Genistein (Isoflavonoid)	Soybean	Skin, Lung, Prostate, Breast, Liver, Gastrointestinal tract, Pancreas, Bladder and other cancers	Demonstrated	Demonstrated
Resveratrol (Phytoalexin)	Grape, red wine	Skin, Lung, Prostate, Breast, Liver, Gastrointestinal tract, Pancreas, Bladder and other cancers	Demonstrated	Demonstrated
Curcumin (Polyphenol)	Turmeric	Skin, Lung, Prostate, Breast, Liver, Gastrointestinal tract, Pancreas, Bladder and other cancers	Demonstrated	Demonstrated
Lycopene (Carotenoid)	Tomato	Skin, Lung, Prostate, Breast, Liver, Pancreas, Bladder and other cancers	Demonstrated	Demonstrated
Luteolin (Flavonoid)	Celery, green pepper, peppermint	Skin, Lung, Prostate, Breast, Liver, Pancreas, Bladder and other cancers	Demonstrated	Demonstrated
Indole-3-carbinol (Glucosinolate)	Cruciferous vegetables	Skin, Lung, Prostate, Breast, Liver, Gastrointestinal tract, Pancreas, and other cancers	Demonstrated	Demonstrated
Sulfuraphane (Isothiocyanates)	Cruciferous vegetables	Skin, Lung, Prostate, Breast, Liver, Gastrointestinal tract, Pancreas, Bladder and other cancers	Demonstrated	Demonstrated
Silymarin (Flavonoid)	Milk thistle	Skin, Lung, Prostate, Breast, Liver, Bladder and other cancers	Demonstrated	Demonstrated
Apigenin (Flavonoid)	Parsley, celery, lettuce	Skin, Lung, Prostate, Breast, Liver and other cancers	Demonstrated	Demonstrated
Organosulfur compounds	Garlic, onion	Skin, Lung, Prostate, Breast, Liver and other cancers	Demonstrated	Demonstrated
Anthocyanins (Flavonoid)	Pomegranate, blueberries, cherries, raspberries	Skin, Lung, Prostate, Breast, Liver and other cancers	Demonstrated	Demonstrated
Delphinidin (Anthocyanidin)	Pigmented fruits and vegetables such as pomegranate, strawberry	Liver and other cancers	Demonstrated	Not-Demonstrated
Lupeol (Triterpene)	Mango, olive, grape, orange, strawberry	Skin, Prostate, Breast, Liver and other cancers	Demonstrated	Not-Demonstrated
Capsaicin (Vanilloid)	Chili pepper	Skin, Lung, Prostate, Breast, Liver, Gastrointestinal tract, Pancreas, Bladder and other cancers	Demonstrated	Demonstrated

* This list provides only selected agents.

our approach, we are attempting to identify the defects at genomic and proteomic levels through which particular cancers could occur in humans. We then need to establish the signature of defects in the individuals for whom chemoprevention is sought. Following the outcome of the first step, in the next two steps of the approach, a scientist with chemoprevention expertise could custom design a cocktail using agents derived from an armamentarium of diet-based substances which could ameliorate the biochemical defect(s) that result in the process of carcinogenesis. Regular follow up might be needed to ensure that aberrant expression of genes and proteins that resulted in cancer onset or development is being repaired. Keeping this three-step approach in mind, novel agents and targets are constantly being developed worldwide. Since this approach is relatively inexpensive, simple to use, and possibly non-toxic, studies to assess its role in cancer prevention in preclinical settings followed by clinical trials will be important. I believe that in the years to come, the greatest gain in reducing the human cancer burden will come from chemoprevention approaches. We need to develop strategies to make it work for the human population.