

# Indiana University Cancer Center Newsletter

February 2000

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***In the spotlight.....***

## **Pamela Crowell, Ph.D.**

Thanks to two of my college science professors, I am telling you today about my career in cancer research. At Augsburg College, a private liberal arts college in Minneapolis, I was a typical premed student, interested in biology, chemistry, and medicine, but thinking only of medical school after college. Two of my science professors encouraged me to consider graduate school as well, which I did. I actually declined a place in the University of Minnesota Medical School in order to attend graduate school in the Biochemistry department at the University of Wisconsin-Madison, and I have never looked back.

As a graduate student in the lab of Alfred Harper, I studied the regulation of skeletal muscle branched-chain keto acid dehydrogenase by diet and hormones. I combined classical approaches of altering the dietary protein content and measuring effects on enzyme activity with a molecular approach, namely measuring diet and hormone effects on the post-translational regulation of the enzyme by phosphorylation. At a FASEB meeting, I met Bob Harris, whose branched-chain keto acid dehydrogenase work I had followed with great interest. Little did I know that I would end up working on the same campus as the Harris lab!

For my postdoctoral research, I chose the field of oncology, and worked in the lab of Michael Gould at the University of Wisconsin-Madison on mammary cancer pharmacology. I was drawn toward cancer research for several reasons: to understand the intriguing process by which normal cells turn cancerous; to do research in a field with clinical relevance; and to carry on the fight against breast cancer that my aunt had lost ten years before. All of my projects in the Gould lab centered around *d*-limonene, a naturally occurring monoterpenoid found in orange peel oil. Gould and others had reported that limonene had chemotherapeutic and chemopreventive activity with very low toxicity in rat mammary cancer

models. We wanted to understand its metabolism and mechanism of action in more detail. For my first project, I did a structure-activity study among several monoterpenoids, testing their ability to inhibit DMBA-induced rat mammary cancer. I became well acquainted with mammary cancer by palpating 300 tumor-bearing rats with 12 mammary glands each several times a week! We did find that all of the monoterpenoids we tested--limonene, carveol, uroterpenol, and sobrerol--were effective antitumor agents. We also identified the circulating metabolites of limonene in rats and then in human subjects using gas chromatography, IR, and mass spectrometry analysis. To address the mechanism of action of limonene, we tested the hypothesis that it would inhibit the post-translational modification of proteins by prenylation. This hypothesis was based on the new finding that the oncoprotein Ras was prenylated, and on the fact that limonene is a chemical cousin to the farnesyl prenyl group that is found on Ras. We found that limonene and its metabolites inhibit protein prenylation, including that of Ras, in several cell types. Our structure-activity study with about 35 monoterpenoids revealed that perillyl alcohol was among the most potent in the inhibition of cell growth and protein prenylation.

At that time, my husband Dring accepted a faculty position in the Biology department at IUPUI, and we moved to Indianapolis. I did a second postdoc in the Cancer division at Eli Lilly, learning molecular biology in the lab of Mei Lai. I cloned the human farnesyltransferase gene (farnesyltransferase catalyzes the prenylation of Ras) and measured its expression in human colon carcinomas, but found no relationship to Ras mutation status. At Lilly, I also had a wonderful opportunity to learn about the whole process of drug development. Then, it was time to seek a faculty position. The IUPUI Biology department had 3 openings, and hired me in 1993.

In my new research program, I wanted to continue studying the efficacy and mechanism of action of monoterpenoids. Their efficacy in a wide variety of rodent cancer models, low toxicity, and unique mode of action (i.e. distinct from agents that inhibit DNA synthesis or mitosis) warranted further research. I searched for cancer model systems that would be appropriate for these studies, and narrowed it down to pancreas, prostate, and ovary. I decided on pancreas, since survival rates from this cancer were poorer than any other cancer type, and new therapeutic strategies were urgently needed. In addition, among human cancers, pancreatic cancers had the highest frequency of K-Ras oncogene mutations, and we knew that monoterpenoids could inhibit Ras prenylation. Very good hamster pancreatic cancer models and matched malignant vs. nonmalignant cell lines were available for these studies as well. So, my first task as a faculty member was to belt out an NIH R29 grant application in which I proposed to test the hypotheses that perillyl alcohol would have chemotherapeutic activity in a Syrian golden hamster pancreatic cancer model system, and that the mechanism responsible was the inhibition of Ras prenylation. NIH funded the R29 the first time around, and we have since learned that half of the hypotheses were right.

We found that perillyl alcohol and other related isoprenoids such as

farnesol, geraniol, and squalene had effective chemotherapeutic activity toward hamster pancreatic ductal adenocarcinomas with only very mild gastrointestinal side effects. The perillyl alcohol result was received with great enthusiasm by the NCI, and it helped to convince the NCI to sponsor clinical trials of perillyl alcohol as a cancer chemotherapeutic agent. On the other hand, Ras farnesylation appeared to have little to do with the antitumor activity of perillyl alcohol in pancreatic cancer, as IC<sub>50</sub> doses of drug from cell growth experiments were ineffective at blocking protein prenylation. We went back to the drawing board for other possible mechanisms. Perillyl alcohol did not affect pancreatic tumor cell proliferation as measured by <sup>3</sup>H-thymidine incorporation into DNA. We therefore turned our attention to apoptosis, and, in more ways than one, hit the jackpot. The American Institute for Cancer Research funded a grant to explore perillyl alcohol mechanisms of action, and we got some interesting results. We found that perillyl alcohol induces apoptosis to a greater extent in malignant than in nonmalignant pancreatic epithelial cells, and the perillyl alcohol-induced apoptosis is associated with higher expression of the proapoptotic protein Bak. Through Steve Williams, I met Pat Loehrer and the entire IU Cancer Center Gastrointestinal Oncology group, and we began discussing plans for a clinical trial. Pat, Tom Howard, Bill Cummings and I are now carrying out a Phase II clinical trial of perillyl alcohol in pancreatic cancer patients. Patients newly diagnosed with pancreatic cancer who are eligible for surgery receive perillyl alcohol in the two weeks prior to surgery, and then tumor tissue is saved for analysis of apoptosis and Bak expression. We will compare the results to those of banked specimens from patients who received no chemotherapy prior to surgery.

A couple of years ago, in collaboration with Biology colleagues Dring Crowell and Steve Randall, we did expression cloning to identify novel human prenylated proteins. We successfully identified two prenylated, oncogenic PRL/PTPCAAX tyrosine phosphatases, and secured an NIH RO1 to investigate the role of prenylation in phosphatase function and oncogenicity. Now, our research focus is gravitating toward combination chemotherapy of pancreatic cancer. For example, my graduate student Dean Wiseman has found that simultaneous administration of perillyl alcohol and Gemcitabine, a cytotoxic nucleotide analog currently used for pancreatic cancer chemotherapy, is more effective at inhibiting cultured pancreatic tumor cell growth than is either drug alone. Dean is examining drug combination effects on the cell cycle and apoptosis, and he is testing different dosing schedules. We are anxious to try this drug combination in the hamster model, and, if it works, begin plans for clinical trials. I feel fortunate to be a member of the IU Cancer Center, where a translational research scheme such as this can quickly become reality. We are also collaborating with Chris Sweeney, Mark Marshall, and Michele Yip-Schneider to test combinations of COX-2 inhibitors with Gemcitabine.

### **Lance Armstrong Foundation Makes Awards**

Brian Giesler, Ph.D., Assistant Scientist, School of Nursing and Medicine,

whose proposal entitled "Long-term Quality of Life of Testicular Cancer Survivors and their Spouses Following Chemotherapy" was recently funded by the Lance Armstrong Foundation to provide a more rigorous assessment of quality of life in the populations affected by testicular cancer. Without the information that this study was designed to provide, patients cannot make informed treatment decisions on the basis of quality of life considerations. Moreover, without a comprehensive understanding of long-term quality of life, health care professionals will be unable to administer effective interventions to relieve the psychological and physical burdens experienced by testicular cancer survivors and their loved ones. Kent Robertson, M.D., Ph.D., Associate Professor of Pediatrics, has also received a one year award from the Lance Armstrong Foundation for his project entitled "AP Endonuclease (APE/ref-1) in Testicular Cancer." This project, that begins January 1, 2000, entails examining the relative expression of APE/ref-1 in good prognosis and high risk testicular cancer/germ cell tumors to determine whether APE/ref-1 expression is a marker for aggressive disease or predictive of therapeutic outcome. Additionally, he will be characterizing the molecular basis of APE/ref-1 protection of germ cell tumors from therapeutic agents.

## **Dr. Quilliam Receives American Cancer Society Grant**

Lawrence A. Quilliam, Ph.D., Assistant Professor, Department of Biochemistry and Molecular Biology, has received a 3 year grant from the American Cancer Society entitled "Role of M-Ras in cellular transformation". Ras proteins are frequently mutated in human cancers and contribute to aberrant cell growth and transformation. Dr Quilliam's group recently identified a Ras related protein that is referred to as M-Ras or R-Ras3 due to its initial isolation from a muscle cell library and closest homology to R-Ras (a protein involved in apoptosis and cell attachment). They have found M-Ras protein to be expressed in many tissues and cell lines and to mimick several of the biological properties of Ras. For example, it induces morphological transformation of NIH 3T3 fibroblasts and inhibits the differentiation of cultured muscle cells.

The ACS grant is to support studies to 1) determine the upstream regulatory events leading to M-Ras activation. This will enable them to identify the physiological hormones/growth factors that utilize M-Ras; 2) to address what unique downstream signaling pathways are regulated by M-Ras and might be targeted to block its action(s); 3) to address the involvement of M-Ras in cell motility and invasion; and 4) to screen human tumor samples for activating M-Ras mutations. The initial focus will be on ovarian tumors in collaboration with colleagues at the Fox Chase Cancer Center

## **New Faces**

David Seitz, M.D., Professor of Medicine, joins the Division of Hematology/Oncology and will see patients in the GI multi-disciplinary

clinic. In addition, he will staff the Wishard Oncology Clinic. Dr. Seitz, formerly with Eli Lilly, brings with him his interests that include developing a clinically annotated human tissue bank to facilitate the investigation of potential prognostic and predictive markers in patients with cancer or who may be destined to acquire the disease. He is also interested in the application of pharmacodynamic endpoints in early phase clinical trials. Dr. Seitz's office is located in the Cancer Pavilion, Room 459. He can be reached through his secretary at 278-7418 or [dseitz@iupui.edu](mailto:dseitz@iupui.edu).

## **Seminars/Conferences/Meetings**

Remember a listing of seminars/conferences/meetings can be found on the Indiana University Cancer Center web page under Seminars and Conferences.

Web Page Address

<http://www.iupui.edu/~iucc/>

If you have a conference, seminar or meeting that you would like posted please contact:

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