

Indiana University Cancer Center Newsletter

November 1999

Special points of interest:

- *In the Spotlight*-Johnny He, Ph.D.
- Harikrishna Nakshatri, Ph.D. Receives American Cancer Society Grant
- Pamela Crowell, Ph.D. Awarded an American Institute for Cancer Research Grant
- Seminars/ Meetings/ Conference Schedule now located on the Web Page

In the spotlight.....

Johnny He, Ph.D.

Hi, everyone!

I'm Johnny He, Ph.D., an Assistant Professor in the Departments of Microbiology and Immunology and Medicine and a member of the Walther Oncology Center.

About myself. I'm a quiet, ordinary guy. I consider my life of the past 34 years very exciting, and adventurous in some senses. I grew up in the city of Chengdu in the People's Republic of China. I love my hometown very much. Chengdu, the capitol of the largest province Sichuan located in the Southwestern part of China, is famous for a variety of Sichuan-style (spicy) Chinese dishes. I spent my early 22 years there. I was accepted into Sichuan University, one of the top ten universities in China. Four years later, I graduated with a B. S. degree in Biochemistry. I was assigned a job as an administrative assistant to the President of Sinica Academia at Chengdu Branch. It is at that time when I came to understand my real interest, i.e., being a biological scientist. So I decided to apply to the graduate school after three years on that job. In the fall 1986, I was admitted into the Microbiology Graduate Program at Beijing Agricultural University. I graduated with the M.S. degree in 1988, one year earlier than expected. Then, I was assigned again a job for the next two years as a research assistant in the Bureau of State Land Management and Planning, studying the potential effects of the Three-Gorge Dam on the macro-agriculture and environment along the Youngtze River.

Since China opened up to the outside world in the early 1980's, there has been more and more college graduates moving overseas. To be honest, I'd never thought about that possibility until I obtained my master's

degree. This was because I had a good job, and on the economic side I had an upper-middle class income, although it was not comparable to that in the States. The desire for a better graduate education made me change my mind about coming to America. In the spring of 1990, I was admitted to the Ph.D. program with a graduate fellowship at New York University (NYU). I was finally boarding a flight to New York city to pursue a new life seven years after I graduated from college.

I don't think I can talk about myself without mentioning two of my previous mentors who have greatly inspired me in many ways. One is my M.S. advisor Dr. Liazhu Liu at Beijing Agricultural University, and the other my Ph.D. advisor Dr. Philip Furmanski at NYU. It is them who taught me to work hard, and to think critically, the skill that I am still working on. It is them who reminded me again and again that it's okay to have disagreements when dealing with science. It is also THEM who showed me that being a scientist is a matter of combined passion, devotion, and sacrifice. I consider myself so lucky to have had the opportunity to work with them.

About my research My Ph.D. thesis was on the study of structure-function relationship of lactoferrin, a natural defense human milk protein in which Dr. Furmanski has had a long-time interest. One of the most significant findings from my Ph.D. research was the elucidation of the specific DNA binding property and the potential physiological significance of lactoferrin, which was later published in *Nature*. These results revealed the existence of a new class of secreted (paracrine) transcriptional activators involved in cell-cell communication. Shortly after my thesis defense I was busy looking for a postdoc position.

In June 1994, I accepted a postdoctoral position at Aaron Diamond AIDS Research Center, Rockefeller University, working on HIV-1 accessory gene Vpr. Among the findings from my work was that Vpr acted as a potential inhibitor of p34cdc2 kinase and caused cells cycle arrest at G2/M phase (published in *Journal of Virology*, one of the most cited papers in 1996). These results provided new insights into the biological functions of HIV-1 Vpr, and opened a new avenue for Vpr research. Eight months later I suddenly found myself in a situation which required me to change my research, due to the unexpected funding problem of my sponsor.

I then began to think more what I would do after I finished my postdoc training. People also suggest to me that postdoc research basically "shapes" and offers the transition to an independent research career. With these in mind, I decided to pursue my interest in AIDS pathogenesis in the central nervous system (CNS). I moved to Dana Farber Cancer Institute, Harvard Medical School in May 1995 to work in Dr. Dana Gabuzda's laboratory. A majority of AIDS patients experience HIV infection of the CNS and exhibit a variety of neurologic disorders. However, there was little known at that time as to the molecular mechanisms of HIV pathogenesis in the CNS. Among the work that I accomplished during my two-year tenure there were that chemokine receptor CCR3 is a major co-receptor for HIV infection in CNS (published in *Nature*, one of most cited papers in 1997), and that HIV infection activates proto-oncogene c-kit, a tyrosine kinase receptor which leads to astrocyte apoptosis (published in

PNAS).

I joined the faculty of the Department of Microbiology and Immunology in October, 1997. Our long-term objective is to identify mechanisms of HIV-1 infection and pathogenesis in the CNS that can be potentially served as therapeutic targets. As a logic extension of my postdoc research, one of the research directions has been focused on mechanisms of HIV entry and pathogenesis in astrocytes. Microglia/macrophages are the major target cells for HIV infection in the CNS. Our group as well as others have found that HIV also infects astrocytes, but to a lesser extent. Astrocytes take up more 20% of the cell volume of gray matter. Astrocytes play a very important role in not only providing nutrients but also maintaining a homeostatic microenvironment for neurons. Thus, it is highly conceivable that dysfunction of astrocytes by direct HIV infection could potentially contribute to neurological diseases. Our current efforts are emphasized on the entry pathway of HIV virus into these cells and effects of HIV expression on astrocyte survival.

The other ongoing research in my laboratory is dealing with the molecular mechanisms of neuronal apoptosis induced by HIV infection. In HIV-infected individuals, it has been shown that it is the neuron death that eventually causes AIDS-associated dementia, which develops in 20-25% adult and more than half of pediatric AIDS patients. However, neurons are rarely infected by HIV virus. A number of soluble products and viral proteins secreted from HIV-infected cells (microglia/macrophages and astrocytes) have been proposed as indirect mechanisms involved in the induction of neuronal apoptosis. Our recent studies showed that HIV-1 Tat, a diffusible viral protein is taken up into neurons by a low-density lipoprotein receptor-related protein (LRP)-mediated endocytic pathway. Importantly, Tat binding to LRP potently inhibited internalization and degradation of neurotoxic LRP physiological ligands including α 2-macroglobulin, apolipoprotein E4, and amyloid precursor protein and subsequent b-amyloid peptide. In this study, we propose a model in which HIV-1 Tat affects neuronal function through a pathway involving disruption of metabolic balance of physiological LRP ligands. Our studies also raise the possibility that HIV-induced neuropathology and other neurodegenerative diseases, such as Alzheimer's disease, may share a common final pathway that eventually leads to dementia.

We are also interested in examining the molecular mechanisms of HIV gene expression in brain cells. For example, we are attempting to determine what host factor(s) are responsible for the abortive (restricted) expression of HIV in astrocytes. Elucidation of restriction mechanisms in astrocytes may shed light on regulation of HIV gene expression, and provide new strategies for developing anti-HIV therapeutics. In addition, we are also continuing our efforts on the development of HIV-2-based lentiviral vectors for gene delivery. I anticipate these efforts will be expanded in my laboratory in the near future.

In case you wonder "what do I think about my job after two years?", the short answer is "I love it", for two major reasons. One is the excellent environment in which I feel free, as a junior faculty, to initiate any research project I wish. On the other hand, I have received the needed

support to establish my research, although there are not too many AIDS researchers around to interact with. Therefore, I'd like to take this opportunity to thank many of my Micro and Walther colleagues, particularly Dr. Broxmeyer and Dr. Spinola for their help and support.

Naksharti Receives American Cancer Society Grant

Harikrishna Nakshatri, Ph.D., Assistant Professor, Department of Surgery has received an American Cancer Society award for his project entitled **"Effect of Phosphorylation on Resistance to TNF and Tamoxifen"**.

The transcription factor NF- κ B protects cancer cells against apoptosis induced by tumor necrosis factor alpha (TNF α) and chemotherapy. While NF- κ B activity is tightly regulated in normal mammary epithelial cells, we have observed that it is constitutively active in estrogen receptor alpha (ER α)-negative but not ER α -positive breast cancer cells. Increased NF- κ B activity in ER α -negative cells leads to overexpression of anti-apoptotic genes, such as cIAP-2, TRAF1 and Mn-SOD, and may allow these malignant cells to escape the host defense response such as TNF α -induced apoptosis. In ER α -positive breast cancer cells, the transcriptional activity of NF- κ B is reduced. We have discovered that this reduction results from binding of NF- κ B to ER α . Current studies indicate that kinases such as mitogen activated protein kinase (MAPK), protein kinase A (PKA) and p90^{RSK1} may phosphorylate ER α and, under such circumstances, ER α cannot inhibit NF- κ B activity. Because MAPK, PKA and p90^{RSK1} are induced by TNF α , we propose that in cell types where TNF α activated kinases phosphorylate ER α , NF- κ B is transcriptionally active despite the presence of ER α and such cells are resistant to TNF α -induced apoptosis.

Tamoxifen, an anti-estrogen, is the most commonly used treatment for ER α -positive breast cancers. Tamoxifen binds to ER α and inhibits estrogen-induced cell proliferation. It also induces apoptosis. For unknown reasons, tamoxifen-resistant tumors eventually grow in a number of patients. Phosphorylation of ER α is believed to play a role in tamoxifen resistance. We propose that increased NF- κ B activity due to ER α phosphorylation in TNF α -resistant cells also leads to tamoxifen resistance because cIAP-2, a NF- κ B inducible gene, inhibits the activity of tamoxifen-induced caspases (which executes cell death).

Based on these observations, we propose to define the mechanism through which TNF α promotes phosphorylation of ER α in certain stages of breast cancer progression leading to a TNF α -resistant phenotype and to test whether blockade of such phosphorylations overcome resistance to TNF α . We will also determine whether cells that are resistant to TNF α are cross-resistant to tamoxifen. NF- κ B activity, TNF α -sensitivity and tamoxifen-sensitivity of ER α -positive breast cancer cells overexpressing kinases such as MAPK, PKA and p90^{RSK1} will be determined. We will also investigate whether dominant-negative mutants of the above kinases reverse the tamoxifen- and TNF α -resistant phenotype of ER α -positive breast cancer cells. Identification of these individual kinases would be a preliminary

aspect of defining a unique resistance pathway in breast cancer cells that is relevant to the resistance against TNF α , tamoxifen, and possibly chemotherapy.

Crowell Receives American Institute Grant

Pamela Crowell, Ph.D., Associate Professor, Department of Biology has received a one year grant from the American Institute for Cancer Research for her project entitled "***Effects of Chemotherapeutic Isoprenoids on the Mevalonate Pathway***".

Recent studies from our lab have demonstrated that the isoprenoids geraniol, farnesol, and squalene have chemotherapeutic activity in pancreatic tumor-bearing hamsters at doses that cause little toxicity. These compounds are products of the mevalonate pathway, the biosynthetic pathway that produces cholesterol as well as prenylated, cell growth regulating proteins such as Ras and Rho. Yet, the effects of geraniol, farnesol, and squalene on the mevalonate pathway in malignant vs. nonmalignant cells have not been addressed. We propose to compare the effects of these isoprenoids in malignant vs. nonmalignant pancreatic cells on several endpoints of the mevalonate pathway, namely protein prenylation, HMG-CoA reductase activity, and cholesterol synthesis. These studies will shed light on the mechanism by which isoprenoids cause tumor regression with low toxicity.

Seminars/Conferences/Meetings Located on the Web Page

Due to the ever increasing number of monthly seminars/conferences/meetings, you can now find these schedules on the Indiana University Cancer Center web page under Seminars and Conferences.

IU Cancer Center Web Page Address

www.iupui.edu/~iucc/

If you have a conference or meeting that is not posted and you would like it posted please contact:

Elizabeth Parsons (eparsons@iupui.edu)

phone 278-0070 or fax 278-0074

and she will have it added to the web page schedule