

## Bankhead-Coley Cancer Research Program

**Ganju-Krishan, Awtar**

Department of Pathology  
H. Lee Moffitt Cancer Center and Research Institute

2006 Program  
Bridge (1-year project)

**Project Title:** Nuclear Marker Expression in Human Breast Tumors

**Project Summary:** As more and more women undergo mammography, the incidence of Ductal Carcinoma in situ (DCIS), a pre-malignant and localized, abnormal cell growth in breast has increased. As it is important to know certain characteristics of the DCIS that may reflect on its behavior and response to treatment, a trained pathologist examines the biopsy and assigns a grade to the tumor. For a trained pathologist, it is relatively easy to differentiate between a low- or a high-grade DCIS. However, a large proportion of DCIS are neither low- nor high-grade and are labeled as belonging to an "intermediate grade." Studies on patients who had DCIS but were not treated or were found to have DCIS at the time of autopsy, suggest that all DCIS do not develop into invasive breast cancer. On the other hand, for the lack of clear-cut distinction between those at low or high risk of developing invasive breast cancer, all patients with DCIS undergo surgery followed by radiation therapy. Thus, it is clear that due to our lack of knowledge about predictive markers, a large number of patients with DCIS are treated who may not need treatment and never develop invasive breast cancer. Pathologists have tried to identify cellular markers, which could be used to discriminate between patients who may need treatment and others who may survive without need for radiation or hormonal treatment. At present cytomorphologic and immunohistochemical examination of breast tumor biopsies is the only standard method used for grading and diagnosis of breast tumors. Several studies have shown that DCIS that have large nuclei, a high percentage of cells in DNA synthesis, and low estrogen receptor expression may be more aggressive than DCIS that have small nuclei, high receptor expression, and low DNA synthesis. Most of these marker studies have been carried out under a microscope, which is slow and based on examination of a small number of cells. Further concerns are related to lack of reproducibility and observer bias (means two different persons grade the DCIS differently). Thus, there is a need for developing automated procedures that can rapidly identify cells with abnormal (aneuploid) DNA content and quantitate expression of the estrogen receptors and other diagnostic markers more rapidly and in a quantitative manner. The team has developed a high-resolution flow cytometer, which can rapidly measure the nuclear volume, DNA content, and expression of hormone receptors simultaneously. This instrument will be used to analyze DCIS and invasive breast cancer samples from patients who stayed disease free and those who developed invasive breast cancer within ten years of diagnosis. Pathology blocks will be collected from the repository, then sections will be cut for the pathologist to reexamine and digest sections to release nuclei. The team has developed methods that allow for the study of these nuclei for the presence of abnormal DNA content (a marker for cancer), measurement of their volume, and determination of the expression of hormone receptors and proliferation status. This data, which will be collected at the rate of millions of cells a minute, will be analyzed to see if a correlation exists between grade of the DCIS as determined by the pathologist using conventional methods and expression of markers determined by this team. The hope is that this may allow pathologists on our team to refine the grading system for breast tumors and ultimately predict which of the patients are at the risk of developing invasive breast

## **Bankhead-Coley Cancer Research Program**

cancer and thus need radiation and other therapy and which patients with low-grade tumors may stay disease free and not need aggressive therapy.