2010 Research Grant Program Winning Abstract

Immune and Molecular Profiling in Bladder Cancer for Personalized Therapy

By Arnold Chin

My laboratory focuses on understanding how inflammation modulates tumor growth using both animal models as well as human studies. As you will see, the use of BD Biosciences antibodies and reagents will be an integral and critical component to the success of our human studies in two areas: 1) investigating lymphocyte activation in bladder cancer patients by examining phosphorylation of key signaling proteins, and 2) sorting tumor stem cells to assess single cell activation using microfluidic devices in our personalized therapy initiative.

Standard Treatments for Bladder Cancer Patients

Bladder cancer has high social, as well as financial, costs. In the United States, it is the fifth most common cancer, with an incidence of more than 70,000 and mortality of greater than 14,000 per year. Treatments can involve extensive surveillance and radical surgery with considerable morbidity, making it the single most expensive cancer in the United States, estimated at $3.7 billion per year. The standard treatments for non-muscle invasive disease are complete transurethral resection followed by adjuvant intravesical administration of Bacillus Calmette-Guerin (BCG), which can decrease recurrences by 50 to 70%. For advanced disease, radical cystectomy with lymph node dissection provides optimal therapy. Neoadjuvant chemotherapy improves survival by about 5%; however, there are no clear pre-treatment predictors.

Determining Immune Activation in Bladder Cancer Patients

We are interested in correlating the inflammatory microenvironment with clinical features such as tumor grade, stage, metastasis, and survival. The presence of tumor infiltrating T lymphocytes has been linked with favorable outcomes in ovarian and colon cancer. However, based on our animal models, we believe that not only presence of T cells, but also negative regulatory factors such as T regulatory cells and myeloid-derived suppressor cells, are important in distinguishing pro- and anti-tumor immunity. An archived bladder cancer cohort of 350 patients between 1985 and 1995 at UCLA has been constructed on a tissue microarray. We will initially stain for anti-CD8+, anti-CD45RO, anti-Foxp3+, and anti-CD11b+, and correlate to tumor grade, TNM stage, lymphovascular invasion, and survival.

Subsequently, we will investigate lymphocyte activation by BD Phosflow™ analysis on contemporary patients prior to surgery. We will compare lymphocyte activation and the tumor microenvironment before and after BCG treatment from single patients who have ultimately failed BCG therapy. We will also compare lymphocyte activation before and after BCG treatment between patients who either responded to or failed BCG treatment. CD8⁺ lymphocyte activation will be examined in peripheral blood mononuclear cells by assessing phospho-Stat1 staining following potentiating by IFN-gamma, and phospho-Stat3 following potentiating by IL-10. A second subset will examine these parameters in
patients undergoing radical cystectomy.

**Personalized Therapy Initiative**

Numerous targeted therapies consisting of small compounds or monoclonal antibodies have been developed, although none have been approved for bladder cancer. In light of the molecular heterogeneity of bladder cancer, we predict differential responses to targeted therapies. By testing tumors of individual patients to sensitivity of known targeted agents and correlating with specific molecular profiles, we will provide the foundation to individualize treatments for bladder cancer patients and potentially improve screening of patients for clinical trials to accelerate development of novel therapies.

Recent evidence has suggested the existence of CD44^+CK5^+ primitive bladder cancer cells or cancer stem cells. A microfluidic platform using a technology called microfluidic image cytometry (MIC) has been used to quantitate single-cell proteomic analysis of EGFR, PTEN, phospho-Akt, and phospho-S6 in as few as 200 cells. In collaboration with our Nanosystems Institute, we will sort CD44^+ cells by flow cytometry into microfluidic chambers for staining of EGFR, ERK, and phospho-AKT pathway activation. In separate chambers, these cells will be tested against mTOR inhibitor temsirolimus, tyrosine kinase inhibitors sunitinib and surafenib, anti-VEGF antibody bevacizumab, anti-EGFR antibody cetuximab, or EGFR inhibitor gefitinib. A fluorescent microscope with specialized software will quantify staining as well as viability by DAPI signals.

**Impact of Studies**

Our goals for these projects will impact the basic understanding of inflammation in cancer, provide prognostic information based on lymphocyte activation or dysfunction in bladder cancer, and will form the basis of understanding the relationship among bladder cancer stem cells, molecular signatures, and sensitivity to targeted therapies. Ultimately, this will lead to individualized tailoring of therapeutic decisions based on an efficient, high-throughput analysis and further the understanding of genetic changes to bladder cancer progression. Early in the career it is difficult to establish the laboratory, generate preliminary data, and stay on track with the long term objectives. This BD Biosciences Research Grant will be an important step in reaching these goals.

The BD Biosciences Research Grant Program aims to reward and enable important research by providing vital funding for scientists pursuing innovative experiments to advance the scientific understanding of disease.

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