GRANT SNAPSHOT

2008 Pancreatic Cancer Action Network Pilot Grant

Grantee:	Pinku Mukherjee, PhD
Institution:	Mayo Clinic, Scottsdale, AZ
Project Title:	*Development of Immune-Modulating Therapies Delivered Directly to the Pancreatic Tumor Site*
Award Period:	July 1, 2007 – June 30, 2008 (No-Cost Extension: August 31, 2008)
Amount:	$60,000

Biographical Highlights

Dr. Mukherjee received her PhD in Immunology from the University of London in the United Kingdom and has been an Assistant Professor at Mayo Clinic College and Director of Cellular Immunology in the Department of Immunolgy, Mayo Clinic. She recently joined the University of North Carolina in Charlotte as an Irwin Belk Professor of Cancer Research in the Department of Biology. Her previous professional involvements include serving as a Senior Research Associate at Pennsylvania State University; Senior Scientist at Indiana University Medical Center; and Associate Consultant at Mayo Clinic College of Medicine. Dr. Mukherjee's research focuses on the development of immunotherapeutic strategies for pancreatic cancer and immune tolerance associated with pancreatic cancer. She has served as a reviewer for the National Institutes of Health.

Project Description

Cancer vaccines present an attractive treatment alternative for pancreatic cancer. They can target the tumor site directly and have fewer side effects. Plus, by generating immune memory against tumor specific proteins, they can prevent metastasis and recurrence of the disease. So far, however, this has not been possible because tumors have adopted ways to successfully escape recognition and killing by the immune cells. Several known agents that can reverse immune escape have previously been tested with modest clinical responses because the agents were administered systemically throughout the whole body and may have never reached the tumor site. In the funded project, novel immune-modulating agents will be targeted directly to the pancreatic tumor site using a tumor-specific MUC1-antibody as a carrier. The study hypothesizes that a robust anti-tumor response along with a strong memory response will be generated when MUC1/KRAS-based vaccine is combined with novel immune modulating agents that are delivered directly to the pancreatic tumor. The hypothesis will be tested using an animal model that develops spontaneous pancreatic cancer and mimics the human disease. Results could provide guidance on how to target other agents to the pancreas tumor site and help develop a new combination modality for the treatment of localized and disseminated tumors.

Results/Outcomes

The most rewarding outcomes relate to the development of the appropriate mouse model, referred to as the PDA.MUC1 mice; the creation of a tumor-specific MUC1 monoclonal antibody conjugated to immune modulators such as CpG-ODN, celecoxib, or 1MT; and the generation of several primary pancreatic cancer cell lines. Treatment of the PDA.MUC1 mice with MUC1 vaccine + celecoxib + low-dose gemcitabine showed tremendous promise. Based on the preclinical results, a clinical trial has been designed and pre-IND meetings with the FDA have been held.

The data with the conjugated MUC1 antibody show the following striking results: (1) conjugated MUC1 antibody recognizes and binds MUC1-expressing human pancreatic tumors of various stages with high efficiency, suggesting that the antibody can be used to target all stages of pancreatic tumors *in vivo;* and *(2)* intratumoral injection of CpG-conjugated MUC1-antibody induced a significant reduction of primary and secondary pancreatic tumor burden in the mice. Antibody-dependent cellular cytotoxicity (ADCC) was determined to be the main mechanism of action, mediated by CpG-activated NK cells. MUC1 antibody conjugated to 1MT or celecoxib shows much less of an antitumor effect by itself and has to be combined with the MUC1 vaccine to induce a synergistic anti-tumor response. Memory responses were only observed when the conjugated antibody was combined with a MUC1 vaccine.

Finally, analysis of Stage 0, 2, 3, and 4 pancreatic cancer tissue and blood (obtained from the Mayo Clinic Biospecimens Registry) clearly indicated that the circulating levels of pro-inflammatory factors, such as PGE_2 and VEGF, are significantly increased with stage. However, MUC1 levels, although significantly higher than age-matched normals, did not increase with stage. Intra-tumoral levels of immune-suppressive cells, such as the T-regulatory cells and myeloid suppressor cells, increased significantly with stage, proving a highly immuno-suppressive microenvironment that is non-conducive to immune intervention. The increase in these immunosuppressive factors correlated with increased PCNA staining, signifying highly proliferative tumor cells.

Lessons Learned

The biggest challenge was the generation of enough triple transgenic mice in a timely fashion for a one-year project. Determination of the correct route of administration is critical. Systemic injections of the antibody were not as effective as intratumoral injections. The importance of activating the innate immune system should not be neglected. This evidence suggests a role for ADCC as a therapeutic mechanism of action of monoclonal antibodies against tumor cells. Moreover, enhanced ADCC activity could induce tumor burden reduction when an immune-modulating agent is conjugated to the tumor-specific monoclonal antibody. Enhanced ADCC by NK cells could induce a variable degree of tumor destruction that conduces to antigen release, favoring the uptake of antigen by antigen-presenting cells, and a tumor-specific T-cell response.

Next Steps

Design a humanized version of the conjugated antibody for clinical trials and determine (a) whether the antibody treatment reverses the immune suppressive tumor microenvironment observed during pancreatic cancer progression, and (b) if pro-inflammatory factors affect the activation of innate immune responses, such as NK cells and ADCC, in pancreatic cancer patients. Dr. Mukherjee plans to study these factors for potential diagnostic and/or prognostic purposes.

Follow-Up Funding

The preliminary data from the funded project was used to write the Project 4 of the Mayo Pancreas Spore. Funding status is pending. The project also has been submitted as an independent RO1.

Publications Related to Funded Project

Tinder TL, Subramani DB, Basu GD, Bradley JM, Schettini J, Million A, Skaar T, Mukherjee P. MUC1 enhances tumor progression and contributes toward immunosuppression in a mouse model of spontaneous pancreatic adenocarcinoma. *J Immunology*, 2008;181: 3116 – 3125.

Basu GD, Tinder TL, Subramani DB, Bradley JM, Arefayene A, Skaar T, De Petris G, Mukherjee P. Progression of pancreatic adenocarcinoma is significantly impeded with a combination of vaccine and COX-2 inhibition. *J. Immunology,* 2008.

Schettini JL, Tinder TL, Mukherjee P. Antitumor activity of NK cells is locally enhanced by CpG-conjugated to a tumor-specific monoclonal antibody. Ninety Ninth Annual American Association of Cancer Research Proceedings, April 2008, #3805.