New research may aid treatment of multiple myeloma patients

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A study led by Robert G. Hawley, Ph.D., professor and chair of the department of anatomy and regenerative biology at the George Washington University (GW) School of Medicine and Health Sciences (SMHS), may help predict which patients with multiple myeloma will respond better to certain treatments. The study, titled "Identification of an ABCB1 (P-glycoprotein)-positive carfilzomib-resistant myeloma subpopulation by the pluripotent stem cell fluorescent dye CDy1," was published in the *American Journal of Hematology* .

Multiple myeloma, the second most common blood cancer in the United States, is an incurable malignancy involving the white blood cells that normally produce antibodies. As the disease progresses, the multiple myeloma cells accumulate in the bone marrow, causing painful bone lesions and preventing normal blood cell production.

"Our hope is that the fluorescent assay we have developed will help physicians monitor the newest treatment option for multiple myeloma patients and determine how well it is working," said Hawley.

Hawley and his team of researchers reported a test that could be used to detect the multiple myeloma cells that survive chemotherapy and are responsible for disease relapse (referred to as tumor-propagating cells).

The researchers tested the hypothesis that the tumor-propagating cells in multiple myeloma exhibit stem cell-like properties that confer resistance to the chemotherapeutic agents used to treat the patients. The team's long-

term goal is to characterize these so-called 'cancer stem cells' in order to develop new targeted therapies that will eradicate the cells and cure the disease. As a first step toward this goal, the research team used a new stem cell imaging dye CDy1, and they isolated pure populations of CDy1-bright and CDy1-dim cells from multiple myeloma cell lines by fluorescence-activated cell sorting (a specialized application of flow cytometry). These multiple myeloma populations were then characterized using RNA-seq 'deep-sequencing' gene expression analysis. Through this next-generation genomics approach, the researchers demonstrated that the CDy1-bright cells did indeed exhibit increased expression of many genes associated with stem cell activity. However, they also noted that the ABCB1 gene, which encodes the P-glycoprotein efflux transporter responsible for multi-drug resistance, was highly expressed in the CDy1-dim population. In functional studies, the investigators determined that dim CDy1 staining was due to the fact that the dye was being efficiently pumped out of the cells by the ABCB1 transporter.

Before the implementation of novel treatment regimens for multiple myeloma over the past decade, ABCB1-associated multi-drug resistance was routinely observed in patients who received conventional chemotherapy containing drugs that are ABCB1 substrates. With this in mind, Dr. Hawley and his colleagues examined new anticancer agents and discovered that high levels of ABCB1 conferred resistance to the second-in-class drug carfilzomib which is currently undergoing evaluation in multiple myeloma clinical trials. Moreover, increased resistance to carfilzomib in sensitive multiple myeloma cells following drug selection was associated with upregulation of ABCB1 cell-surface expression which correlated with increased transporter activity as measured by CDy1 efflux.

Of special note, carfilzomib (marketed under the brand name Kyprolis) recently received accelerated approval by the U.S. Food and Drug

Administration for the treatment of multiple myeloma patients who have received at least 2 prior therapies and whose disease continues to worsen. Therefore, the next phase of the project, which is supported by a pilot research grant awarded in 2012 by The Dr. Cyrus and Myrtle Katzen Cancer Research Center at GW, will be to translate the laboratory findings to the clinic. This work, which will be carried out in collaboration with Imad Tabbara, M.D., professor of medicine at GW SMHS, will involve screening multiple myeloma patients to determine whether the CDy1 assay can help guide treatment decisions or predict which patients will respond better to carfilzomib.

"I first became interested in this subject as a graduate student at the Ontario Cancer Institute in the early 1980's," said Hawley. "However, I became disheartened as a principal investigator in the late 1990's when we were unable to cure the disease in a mouse model using cutting-edge cancer gene therapy, and I stopped working in this area. Despite recent therapeutic advances, multiple myeloma remains incurable. About a year and a half ago, I had a conversation with Robert Siegel, M.D., professor of medicine at GW SMHS and director of the Katzen Cancer Research Center, who encouraged me to enter the field again, and I am really happy that I did."

Hawley's team of researchers includes lead coauthors Teresa Hawley, B.S., director of the GW Flow Cytometry Core Facility, and Irene Riz, Ph.D., assistant research professor of anatomy and regenerative biology, along with Louis DePalma, M.D., professor of pathology and of anatomy and regenerative biology, and Weiqun Peng, Ph.D., associate professor of physics and of anatomy and regenerative biology, together with collaborators Jun Zhu, Ph.D., director of the DNA Sequencing and Computational Biology Core at the National Heart, Lung and Blood Institute of the National Institutes of Health, and Young-Tae Chang, Ph.D., head of the Laboratory of Bioimaging Probe Development at the National University of Singapore Agency for Science, Technology and

Research.

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