



## 2010 Research Grant Program Winning Abstract

### Cancer Stem Cells in Myeloma

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An underlying principle in cell biology is that the cell's genotype drives its phenotype and subsequently, morphology. While this premise holds true for most normal tissues, in cancer, genetic alterations lead to perturbed phenotype and cell morphology making it exceedingly hard to determine the true identity of the cancer cells. Multiple myeloma (MM) is an incurable bone marrow (BM) malignancy of B cell lineage characterized by monoclonal plasma cells (PCs) in the BM and accounts for 19% of deaths from hematopoietic malignancies. MM presents with overproduction of monoclonal immunoglobulin, suppressed hematopoiesis, and lytic bone lesions. Most patients initially respond to therapy, but despite the development of potent new regimens, nearly all relapse and become refractory to treatment; thus the median survival rate remains 3-5 years. These observations indicate that the MM cancer stem cells (MM-CSCs) escape current modes of therapy.

The cancer stem cell (CSC) hypothesis postulates that only a small sub-population of cells can initiate a tumor or cause a relapse. CSCs are quiescent, their proliferation is limited to infrequent cell divisions without differentiation, drug resistant, and exhibit both tumorigenic potential and multipotency, the ability to differentiate into all cell types of a heterogeneous tumor. Since most CSCs never initiate the tumorigenic program, they remain quiescent throughout the life of the individual. Thus, understanding the nature of CSC quiescence will provide an opportunity to eradicate cancer by designing new strategies to prevent its initiation. However, before we can study the biology of the CSCs, we need to assemble the tools to reliably isolate these cells from cell lines and primary tumors. The studies proposed here will define the surface markers expressed by MM-CSCs.

Cell culture systems where cells are grown on the surface of tissue culture plastic, fail to accurately represent the architecture of the tissue, and thus, the complex interactions between cells and their microenvironment. We have recently described a 3-dimensional (3-D) tissue culture model mimicking the extracellular matrix (ECM) microenvironment of the BM where cells self-organize into distinct niches. To recreate the spatial organization of the BM, primary BM mononuclear cells are grown in an ECM scaffold of BD Matrigel™, collagen I, and fibronectin. Utilizing this 3-D model we isolated drug-resistant, self-renewing, multipotent putative MM-CSCs based on their proliferative quiescence (label retention) and combined with the presence of the side population in 3-D culture. Recently, we demonstrated that these cells are tumorigenic in an immunodeficient mouse xenograft model.

Current studies disagree on the identity of the MM-CSC with some evidence pointing to early B cells, some to late stage B cell, some to lymphoblasts, and yet other to PCs. Frequently cancer cells express cell surface antigens seemingly contradictory to their apparent phenotype, thus masking their true identity. The objective of this study is to assemble a comprehensive panel of antibodies specific to various stages of B cell



development to systematically characterize the surface antigens expressed on MM-CSCs. We hypothesize that there exists a unique combination of surface markers defining the MM-CSCs, and that these surface markers can be used to purify MM-CSCs for further analysis.

Aim 1: Characterize the cell surface makers expressed by putative MM-CSCs.

Aim 2: Determine the tumorigenic potential of MM-CSCs purified using the markers identified in aim 1.

To determine the placement of the MM-CSC within the B cell hierarchy, label retaining, Hoechstlow cells sorted from 3-D cultures will be interrogated against a panel of antibodies specific for each step of B cell development (pro-B, pre-B, activated B, memory B, and plasma cells). To take into account aberrations occurring in cancer cells compared to normal cells of B lineage, we will use a broad panel of antibodies in flow cytometry and immunofluorescence experiments to define the MM-CSC signature. The panel will include CD10, CD19, CD20, CD21, CD22, CD27, CD28, CD34, CD38, CD40, CD45, CD56, CD72, CD79, CD80, and CD138 with various combinations of these antigens providing a signature for cells in the B cell hierarchy. The use of a broad panel of B lineage antigens to characterize the MM-CSC will ensure an unbiased analysis of the MM-CSC phenotype. Once the unique combination of the cell surface markers is identified, the tumorigenic capacity of the cells exhibiting the identified phenotype will be validated in the immunocompromised mouse xenograft model.

To complete the studies proposed here we will utilize a number of BD reagents including BD Matrigel and fluorescently labeled antibodies. Aim 1 will require the use of ECM proteins (BD Matrigel, collagen I, fibronectin) to grow primary bone marrow cells and myeloma cell lines in 3-D culture and a panel of antibodies against cell surface antigens listed above. Aim 2 will require Matrigel, collagen I, and fibronectin to reconstruct human BM microenvironment in a xenograft mouse model. Antibodies will be required to sort MM-CSCs based on the expression pattern of CD antigens identified in aim 1. To test the tumorigenic potential of MM-CSCs and the ability of the resulting tumors to generate lytic bone lesions, sorted MM-CSCs will be co-injected with ECM proteins into the mouse tibia. Thus, BD reagents will be crucial to achieve the goal of this study: to identify a unique surface expression signature associated with the MM-CSC phenotype.

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