

Career Development Candidate Leukemia Spore

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Leukemia SPORE Career Development Award

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Project Proposal: The Significance of DNA Methylation as an Epigenetic Factor in the Development and Progression of Polycythemia Vera.

Background:

Polycythemia vera (PV) is the most common myeloproliferative disorder with the yearly incidence of 2.8 per 100,000 people. A slightly higher prevalence has been reported in males (1.4:1). PV is a trilineage hyperplasia with a clonal expansion of myeloid cells of erythroid, myelomonocytic, and megakaryocytic lineages, erythrocytosis being the most prominent clinical manifestation of PV. The disease is associated with a significant morbidity and mortality, including thrombotic and/or hemorrhagic events, and a risk of an evolution into myelofibrosis and leukemia. Specific factors causing PV have not yet been identified. An acquired mutation giving a proliferative and/or survival advantage to a single hematopoietic stem cell is presumed to be a primary event, however, the molecular basis of PV remains unknown. Chromosomal abnormalities are present in about 25% of patients; none of them was found to be PV-specific. Search for mutations in the molecules involved in cellular growth factor signaling and cell survival has been negative so far. Methylation of cytosines in the palindromic CpG sites clustered in the gene promoter regions is an important mechanism of epigenetic gene silencing, and acts as one possible mechanism of tumor suppressor inactivation in cancer. It is reasonable to assume that epigenetic changes are also involved in the pathogenesis of PV; however, experimental data are scarce.

Objective/Hypothesis:

We propose to investigate the role of cytosine methylation in CpG islands associated with the promoter regions of genes in the development of PV and in its transformation to myelofibrosis and leukemia. We will test the hypothesis that methylated promoter-associated CpG islands found in PV cells may represent early events in the transformation.

Study Design:

As a first step in testing our hypothesis we will employ a genome-wide screening test (Methylated CpG Island Amplification coupled with Representative Difference Analysis, MCA-RDA) for the detection of methylation of CpG islands in gene promoter regions in PV patients. We next will develop high throughput bisulfite pyrosequencing assays for a thorough analysis of the methylation status of CpG islands recovered by MCA-RDA in blood cells and bone marrow from PV patients. In order to explore the hypothesis that methylation of promoter CpG islands detected by MCA-RDA represents early changes in transformation, bone marrow DNA samples from patients with myelofibrosis and acute myeloid leukemia (AML) evolving from PV will be assayed by bisulfite pyrosequencing and results compared to those in non-transformed patients. To determine whether methylation patterns in myelofibrosis and AML evolved from PV are unique to those patients, we will compare them to that of myelofibrosis and AML evolved from essential thrombocytosis, as well as to that of essential myelofibrosis and primary AML. We shall determine whether the aberrant methylation detected by MCA-RDA and bisulfite

pyrosequencing correlates with gene silencing by measuring the levels of specific mRNAs by quantitative real time RT-PCR.

Relevance:

The characterization of epigenetic changes in PV will contribute to a better understanding of the pathogenesis and evolution of this disease. Assessment of methylation of genes involved in transformation of PV may be developed as a diagnostic test for the detection of early transforming changes in PV patients. Finally, attempts for their reversal might open new possibilities for the treatment of PV with DNA-demethylating drugs and other agents affecting the chromatin structure.