Microenvironment a main driver of aggressive multi-lineage leukemia disease type

June 9 2008

Research led by scientists at Cincinnati Children's Hospital Medical Center has revealed new clues into what causes different types of a particularly aggressive group of blood cancers known as mixed lineage leukemias (MLL) and how the disease might be treated, according to a study in the June 9 issue of *Cancer Cell*.

"We document early biological processes where human leukemia stem cells can be altered to form a particular type, or lineage, of leukemia by the factors they are exposed to in the microenvironment of blood-forming tissues," said James Mulloy, Ph.D., a researcher in the division of Experimental Hematology/Cancer Biology at Cincinnati Children's and the study's corresponding author. "These new details about molecular events associated with MLL, and the new mouse model we developed for the study, will allow testing of novel therapeutic strategies for MLL patients. They will also yield information that may be directly translatable into clinical interventions."

Leukemia is the most common blood cancer and includes several diseases, according to the National Cancer Institute. The four major types are acute myeloid leukemia (AML), acute lymphoid leukemia (ALL), chronic lymphocytic leukemia (CLL) and chronic myelogenous leukemia (CML).

Mixed lineage leukemia (MLL) results when chromosome mutations

involving the MLL gene fuse with certain partner genes. These so-called translocations result in the MLL gene being rearranged to send instructions to create either AML or ALL. This process can start before birth, and while MLL translocations are associated with 7 percent of AML cases and 10 percent of ALL cases, they are found in a majority of infants with acute leukemia. In some instances the clinical disease is diagnosed within a few months of birth.

Dr. Mulloy and his colleagues discovered that disrupting a protein known to regulate cell growth (Rac1) has potential for curbing MLL. The discovery came about as they focused on the most common fusion partner in MLL, a gene called AF9. Previous research showed patients with MLL-AF9 fusions almost exclusively get AML, have an intermediate to poor prognosis, and that leukemia expressing MLL-AF9 is considered a more aggressive disease resistant to chemotherapy.

Although MLL-AF9 fusion is most commonly associated with AML in people, it is occasionally found in ALL as well. Dr. Mulloy's team programmed human umbilical cord blood cells to express MLL-AF9, resulting in diverse leukemia stem cells capable of transforming into either AML or ALL. The researchers influenced the transformation by altering the growth factor proteins that stimulate the differentiation and growth of blood cells, demonstrating how environmental conditions play a critical role in promoting leukemia progression and deciding disease type.

The researchers then built on this finding by adjusting the cell culture microenvironment to transform lymphoid cells into myeloid cells, as well as myeloid cells into lymphoid, highlighting the adaptability of the leukemia stem cell in mixed lineage leukemia.

"Our findings underscored that while some leukemia stem cells in MLL are diverse and able to transform into different lineages, others remain

committed to a single disease type," Dr. Mulloy explained. "This information, and our ability to successfully develop human-based MLL models in mice, will be very useful in finding further insights into the early molecular events behind poor prognosis in mixed lineage leukemia."

Researchers also experimented with inducing AML or ALL in mice by using the MLL-AF9-expressing human cord blood cells. Although mouse models have been successful for studying leukemia stem cells in MLL-associated AML, their usefulness is considered limited for modeling the lymphocytic and mixed myeloid/lymphoid forms of the disease. The research team overcame this limitation by transplanting human MLL-AF9-expressing cells into two strains of mice. Both strains were bred for severe immunodeficiency (NS), which allowed human cells to be grafted into the mice. One of the strains also contained three human cytokine proteins that control blood cell formation and promote myeloid cell development (NS-SGM3).

NS-SGM3 mice receiving the MLL-AF9-programmed cells all developed AML (acute myeloid leukemia) in five to seven weeks, even when most of the MLL-AF9-expressing cells were lymphoid. The three cytokines in the mice were able to redirect the leukemia stem cells from lymphoid to myeloid. However, in the NS strain of mice, the same cells led to the development of a mix of ALL, AML and acute bi-phenotypic leukemia (ABL). In ABL, at least 20 percent of the cells have indications of both myeloid and lymphoid disease. These findings further demonstrated the importance of microenvironment in determining the lineage outcome of disease, the researchers said.

Relatively little is known about the important molecular events that are downstream of the MLL fusion gene. Previous research indicates that Rac1 – a protein that helps regulate cell growth – has increased activity in mice with AML expressing MLL-AF9. To test the importance of

Rac1's downstream regulatory pathway in human AML expressing MLL-AF9, the research team experimented with a small molecule that inhibits Rac1's activity. They also tested genetic manipulation of Rac. Both interventions prevented MLL-AF9 cell growth and induced programmed cell death (apoptosis), suggesting Rac as a possible therapeutic target in AML involving rearrangement of the MLL gene, according to Dr. Mulloy and his fellow researchers.

"The exquisite sensitivity of the leukemia cells to Rac inhibition indicates that the MLL-AF9-expressing cells have become addicted to this signal, and this pathway is therefore a very good target for future drug development" said Junping Wei, M.D., Ph.D., a researcher at Cincinnati Children's and lead author of the study.

Source: Cincinnati Children's Hospital Medical Center