Major gene study uncovers secrets of leukemia

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Investigators at St. Jude Children's Research Hospital have discovered previously unsuspected mutations that contribute to the formation of pediatric acute lymphoblastic leukemia (ALL), the most common cancer in children. The discovery not only suggests novel methods for treating pediatric ALL, but also provides a roadmap for the identification of unsuspected mutations in adult cancers.

ALL is a tumor in which immature white blood cells that normally develop into immune system cells, called B or T lymphocytes, instead multiply rapidly and overwhelm the normal blood cells the body needs to survive.

The St. Jude team used microarrays, postage-stamp-sized chips that contain DNA fragments, which allowed researchers to investigate more than 350,000 markers called single nucleotide polymorphisms. Single nucleotide polymorphisms are individual variations in the DNA that are spaced across the human chromosomes. Single nucleotide polymorphisms function as flags for researchers, allowing them to detect specific deletions of DNA in a gene or increases in the number of specific genes at a level of detail that was previously unattainable. The St. Jude group used this approach to analyze leukemia samples from 242 pediatric patients with ALL. This identified an unexpectedly high frequency of mutations involving genes that function as master regulators of normal B-cell development and differentiation.

A report on this work appears in the March 7 online edition of *Nature*.

"The results of our study demonstrate that it is possible to significantly speed the identification of the genetic lesions that are the underlying cause of not only ALL, but also many other cancers, including those affecting adults," said James Downing, M.D., scientific director and chair of the Pathology department at St. Jude. He is senior author of the paper.

The study found that 40 percent of patients with ALL had deletions or mutations in one of three so-called "master genes" that control the normal differentiation of immature progenitor cells into mature B lymphocytes.

The researchers found that the "PAX5" gene was most frequently mutated—altered in about 30 percent of patients. These mutations reduced the level of PAX5 protein in leukemic cells or resulted in the formation of PAX5 protein with defective function. Mutations were also found in other genes with important roles in B-cell differentiation including "EBF1" and "Ikaros."

"Although the identification of such a high frequency of mutations in this pathway was surprising, it is important to note that the approach used provides a lower limit of the true frequency of these mutations, since not every gene in this pathway could be accurately analyzed using this methodology," Downing said.

The mutations identified in "PAX5," "EBF" and "Ikaros" are likely to directly contribute to this block in normal lymphocyte differentiation, according to Downing. These genes encode proteins called transcription factors, which orchestrate the expression of a large number of other genes involved in B cell development. Together these genes coordinate the complex changes needed to induce progenitor cells to differentiate into B lymphocytes. In ALL, the leukemic cells fail to differentiate normally and instead remain blocked at an immature stage of

development. Locked in this state, the leukemic cells continue to proliferate, and this continual growth of leukemic cells eventually kills the child.

"The new insights into the differentiation of B cells are extremely valuable," said Ching-Hon Pui, M.D., chair of the Oncology department and American Cancer Society Professor at St. Jude. Pui co-authored the paper. "The more we learn about why progenitor cells get stuck in the primitive, cancerous stage, the more likely we'll be able to design new therapies that eliminate them. That could help us continue our successful efforts to increase the survival rate of ALL."

One potential strategy for eliminating leukemic cells would take advantage of the discovery that mutations in the B-cell differentiation pathway are predicted to prevent progenitors from changing into normally functioning lymphocytes. Normally, the body eliminates differentiated B lymphocytes that have failed to assemble the right genes to make effective antibodies against the specific target they are supposed to attack. However, if these defective B lymphocytes do not differentiate because of the mutations, the body will not recognize them as defective immune cells and destroy them. Instead, these undifferentiated cells continue to multiply, causing ALL.

"If we could design a drug that bypasses the roadblock to differentiation, we could push these cells to become fully mature B lymphocytes," Downing said. "And then the body would recognize them as defective B lymphocytes and destroy them."

Source: St. Jude Children's Research Hospital