Genome-Wide SNP Analysis in Cancer: Leukemia Shows the Way

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The application of novel genetic/genomic technologies to the study of acute leukemia has frequently been a proving ground for such approaches in cancer. Recent development of high-resolution single-nucleotide polymorphism (SNP) arrays allows detailed assessment of the genomes in cancer cells. A recent study by Mullighan et al. uses SNP arrays to assess copy number alterations in a large group of childhood acute lymphoblastic leukemias and demonstrates frequent mutation of genes encoding transcription factors important for B cell development. These studies not only provide information about the multistep development of leukemia, but also demonstrate the potential for this approach in other cancers.

Childhood acute lymphoblastic leukemia (ALL) is the most common malignancy diagnosed in children, representing approximately one-third of all pediatric cancers. Remarkable progress has been made in the treatment of this disease, with approximately 80% of patients now being cured with combination chemotherapy. However, as some 15%–20% of patients relapse after initial therapy, and those that are cured often experience late effects from chemotherapy, there remains a significant need for new therapeutic approaches that target the underlying genetic abnormalities driving leukemia development. The application of newly developed genomic tools to the study of leukemia promises to uncover the genetic lesions important for tumor initiation and progression. Indeed, the study of leukemia, perhaps more than most cancers, has been enhanced by the application of newly developed tools to study genomic integrity. From early studies probing for cytogenetic abnormalities to more recent gene expression studies, leukemia is frequently a cancer where new technologies yield important insight. Such studies have led to the identification of a number of recurrent translocations and other genetic alterations that are currently used to risk stratify patients based on their likelihood of being cured with standard therapy. Hopefully, in the not too distant future, these genetic abnormalities will be targeted with therapeutics that mimic the success of Imatinib in chronic myelogenous leukemia (CML) (Druker et al., 2001).

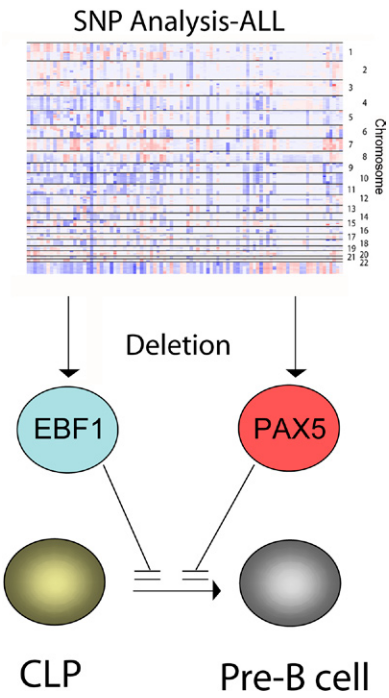

Figure 1. SNP Analysis of B-ALL Identifies Recurrent Mutations in Genes Important for Normal B Cell Development
Mullighan et al. analyzed 242 pediatric ALL cases using genome-wide SNP analysis. Recurrent deletions in B-precursor ALL samples were identified in genes such as *EBF1* and *PAX5* that are known regulators of B cell development. This suggests that perturbation of normal pathways that promote development from common lymphoid progenitors (CLP) to pre-B cells is an important part of the pathogenesis of B-ALL and highlights the power of genome-wide assessment of cancer cells.

A recent study by Mullighan et al. has taken an important next step in the characterization of the genetics of childhood ALL and uncovered new recurrent genetic alterations (Mullighan et al., 2007). In so doing they highlight the power of high-resolution genome-wide assessment for genetic alterations in cancer cells. This study assesses leukemia cell DNA obtained from 242 pediatric patients with ALL through the use of single-nucleotide polymorphism (SNP) arrays that provide the highest-resolution analysis of genomic integrity to date. Global assessment for copy number alterations demonstrates a mean of 6.46 somatic copy number alterations per ALL sample, demonstrating a remarkably stable genome when compared to what is likely the case in other tumors, particularly carcinomas. When analyzed with respect to known chromosomal translocations found in ALL, there was variability ranging from one alteration per genome in cases harboring rearrangement of the *MLL* gene on chromosome 11q23, to 6.8 alterations per genome in cases with *BCR-ABL* translocations, perhaps providing insight into the number of genetic alterations necessary for leu-

kemia development. When analyzed for recurrent alterations, 54 recurrent somatic mutations were identified that involved a number of genes with potential roles in tumorigenesis. While detailed functional studies will be necessary to appreciate the importance of the majority of these alterations, some of the more commonly altered genes, *EBF1* (8 B-ALL cases), *PAX5* (57 B-ALL cases), and *IKZF1*(*IKAROS*) (17 B-ALL cases) play important roles in normal B cell development, thus immediately highlighting a potential role for these genes in the pathophysiology of B-precursor ALL (B-ALL) (Figure 1).

Further characterization of the mutations in genes associated with B cell development supports the importance of these alterations in B-ALL cases. The majority of the cases with *EBF1* abnormalities were monoallelic deletions; however, in one particularly informative case, two populations of leukemic blasts were identified, a relatively immature CD10/CD22^{dim} population and a more mature CD10/CD22^{bright} population. FISH analysis of flow-sorted blasts revealed homozygous deletions of EBF1 in the immature pro-B cell-like population, and hemizygous deletion in the more mature blasts. These data, combined with data from mouse models demonstrating a block in B cell development in the absence of EBF1, are strongly supportive of EBF1 mutations playing a role in the perturbed lymphoid differentiation found in B-ALL (Lin and Grosschedl, 1995).

Moreover, the study showed that *PAX5* is frequently the target of genetic alterations in B-ALL. The most frequent types of alteration identified by SNP analysis were monoallelic deletions of varying size that primarily resulted in loss of function. Further characterization of the *PAX5* gene in other B-ALL cases identified a *PAX5-ETV6* translocation in one case and two novel *PAX5* translocations (*PAX5-FOXP1*, *PAX5-ZNF521*) in other cases. Each of these chimeric genes encodes a fusion protein that retains the DNA-binding domain of PAX5. Thus, the fusion proteins could bind to PAX5 transcriptional targets but would presumably no longer provide normal transcriptional activity. Functional studies demonstrated the fusion proteins competitively inhibit the transcriptional activation activity of wild-type PAX5, thus supporting a dominant-negative role for the fusion proteins in ALL. Finally, novel point mutations that resulted in altered transcriptional activation were identified in some B-ALL samples. These observations, combined with previous studies in mouse models that demonstrate that absence of *Pax5* results in disordered B cell development (Lin and Grosschedl, 1995; Nutt et al., 1999) point to *PAX5* mutations as a frequent participant in the development of human ALL.

The study also identified deletions involving additional genes encoding regulators of B cell development, including *IKZF3(AIOLOS)*, *LEF1*, and *TCF3(E2A)*. Differences in the frequency and type of mutations were observed among various subtypes of ALL with the deletions in B cell development associated genes being most frequent in B-ALL cases with a hypodiploid genome. Also, a relatively high frequency of *PAX5* monoallelic deletions (28%) were found in B-ALL cases with *TEL-AML1* rearrangement. It will be of interest to determine if there is a statistically significant association between particular deletions and specific genetic subtypes of ALL. These data raise the interesting question whether the newly defined genetic alterations cooperate with chromosomal translocations such as *BCR-ABL*, *MLL*-translocations, or *TEL-AML1* during leukemia progression. It is widely believed that previously defined chromosomal translocations are initiating events in childhood ALL (Greaves and Wiemels, 2003). However, detailed epidemiologic studies and sophisticated murine models suggest that multiple genetic events are necessary for ALL development, as has been similarly hypothesized in acute myelogenous leukemia (Gilliland and Griffin, 2002; Greaves and Wiemels, 2003). While studies have identified signaling molecules such as FLT3 or RAS as potentially important in some ALL cases (Armstrong et al., 2003; Liang et al., 2006; Taketani et al., 2004), until this study, candidate cooperating mutations have been lacking in the majority of B-ALL cases. It is interesting to note that the identification of mutant genes in B-ALL is reminiscent of the frequent mutation of *NOTCH1* in T-ALL (Weng et al., 2004). As *NOTCH1* signaling is important for normal T cell development, it appears that perturbation of developmental pathways unique to specific B or T lymphocyte lineages may be a common theme in ALL pathogenesis. Future studies will determine what specific roles the lymphocyte developmental pathways are playing in leukemogenesis, and if these newly defined genetic abnormalities can be used as prognostic markers, or more importantly to develop new therapeutic approaches. Genome-wide analyses of cancer such as this will continue to provide insight into the pathophysiology of human cancer and should provide new opportunities for development of targeted therapies.

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