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Genomic Analysis of Acute Myeloid Leukemia; Potential for New Prognostic Indicators

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Abstract

Purpose: Acute myeloid leukemia (AML) is a heterogeneous group of clonal myeloid malignancies. With a few exceptions, response to treatment is unsatisfactory and prognosis is poor. Studies indicate that specific cytogenetic abnormalities, identified by classical G-banding, correlate with prognosis. These findings advanced the ability to predict outcome and to tailor treatments in AML. These studies also suggested that a more detailed analysis of somatic genomic mutations might extend these advances.

Recent Findings: New technologies, including DNA-arrays and automated sequencing, have improved detection of subtle, acquired genomic alterations. DNA-array based screening approaches permit detection of copy number alterations (CNA) of less than 5 megabase (Mb) in size. Sub-chromosomal copy number neutral loss of heterozygosity (CNN-LOH) can also be detected using approaches that take advantage of single nucleotide polymorphisms (SNPs) in the human genome. However, identification of single nucleotide variants (SNV) in leukemic clones still requires targeted or massive sequencing approaches.

Summary: Recent studies suggest that CNA and CNN-LOH occur frequently in AML. Recurring abnormalities have been identified which may be relevant to disease pathogenesis. However, larger studies will be required to determine the relevance of these alterations to prognostic prediction or therapeutic targeting.

Keywords

acute myeloid leukemia; genome; somatic mutation; uniparental disomy

Introduction

In recent years, there has been increasing interest in using molecular analysis to identify prognostic indicators or therapeutic targets for AML. One approach is to identify somatic genomic alterations in leukemic clones. In previous studies, chromosomal alterations that indicate favorable or adverse prognosis were identified by classical G-banding karyotype analysis. These studies determined that translocations involving the retinoic acid receptor or core binding factor proteins indicate a favorable prognosis, a normal karyotype indicates an intermediate prognosis, and a complex karyotype, or lesions involving the *MLL* gene indicate an adverse prognosis.

Based on the success of these studies, methods were developed to identify sub-chromosomal genomic lesions associated with AML. Using micro-dissection-based comparative genomic hybridization (CGH), chromosomal gains or losses of 5-10 Mb were detected in AML (1,2, 3). This technique was refined using array based CGH which permits detection of CNA of less than 5 Mb (4). The development of specific statistical analysis algorithms (5) led to the use of SNP-arrays to investigate CNA in AML. Use of SNP-arrays also identified CNN-LOH (or acquired uniparental disomy; aUPD) in AML. CNN-LOH may include large regions at the ends of chromosomes (30-90 Mb) or smaller interstitial sequences (2-8 Mb) (6). Such studies have identified recurring areas of CNA and CNN-LOH in AML.

Single bp mutations that are relevant to the prognosis of AML have been identified in a number of studies. Such mutations may worsen prognosis (activating *FLT3* or *KIT* mutations) or improve it (*NPM1* mutations). The development of high throughput automated sequencing techniques suggests the possibility of sequencing leukemia genomes to identify novel single nucleotide variants (SNV) or small genomic duplications. Although potentially a powerful, the current technology requires substantial effort for each sample (7).

Copy Number Alterations (CNA) in AML

Recent studies, using CGH, or SNP-microarrays, have identified a recurring set of acquired CNA associated with AML. The potential significance of these results for the pathogenesis of myeloid malignancy is suggested by the presence of a number of tumor suppressor genes in areas of loss and oncogenes in areas of genomic gain.

Studies of CNA in normal karyotype AML are of interest to further define prognostic groups in this heterogeneous, intermediate prognosis category. In one study, Akagi et al. identified CNA in 24% (9 of 38) normal karyotype AML samples using 250K SNP-arrays (8). Areas of genomic loss included genes encoding tumor suppressors such as *NF1* (17q; encoding Neurofibromin), *EVT6* (12p; encoding Tel) or *CDKN2A* or *2B* (9p; encoding cyclin dependent kinase inhibitor). They also found recurrent gains in chromosome 8 in a region including the *MYC* gene. Consistent with these results, mRNA expression studies documented decrease in Nf1, Tel and Cdki and increase in c-myc. In another study, Walter et al. investigated CNA in 86 newly diagnosed AML subjects, including 40% with normal karyotype (9). They found that 24% of the normal karyotype subjects had CAN (in comparison to 40% of subjects with various abnormalities in karyotype identified by G-banding). These authors found 50 recurrent CNA of less than 5 Mb with losses more common than gains. Regions with leukemia or cancer associated genes were statistically over represented in these studies including 12p, 17q, 5q, 7q and 16q. Amplification of 8q was identified as were amplification of 11q (including the *MLL* gene), 19q and 21q.

AML with complex karyotype is another subgroup for which CNA studies are potentially informative. Determination of chromosomal gains or losses by conventional G-banding is difficult in samples with multiple translocations. Rucker et al. investigated 60 AML samples with complex karyotype using a custom CGH array (10). The found CNA in 100% of samples with losses greater than gains. The most common losses involved 5q, 17p (including *TP53*) and 7q, and the most common gains were in 11q and 8q. Less common losses included 16q, 17q and 18q. In this study, gains or losses of chromosomal material also correlated with altered genes expression in the involved regions. Kim et al studied 19 cases of complex karyotype AML using micro-dissection based CGH (3). They found CNA in 9 of these cases, consistent with the lower sensitivity of this method. However, they also identified most frequent losses in 5q and 17p and most frequent gains including 8q and 11q. Therefore, both of these studies suggest that complex karyotype AML is characterized by increased c-myc and Mll and decreased p53.

Although acute promyelocytic leukemia (APL) has a relatively good prognosis, some patients with APL respond to treatment more poorly than anticipated, despite the absence of additional cytogenetic markers. A group of 47 subjects with t(15;17) APL were studied by Akagi et al. using SNP-microarrays (11). They found that 60% of these subjects had no CNA. Of those with abnormality, 8 of 11 had amplification involving chromosome 8. Losses were less frequent (7 total with only 7q appearing in more than one case). They also investigated *FLT3* mutation status and found activating *FLT3* mutations only in samples without CNA. Therefore, the subjects fell into three groups; 8+, Flt3-mutant, and no other abnormalities. These groups will be of interest for future prognostic studies. Karnan et al. investigated APL subjects using CGH microarrays (12). In this study of 30 APL subjects, 16 were found to have CNA. This included recurrent losses in 1p, 2p, 16p and 17p and gains in 8p, 8q and 13q. In contrast to the previous study, these investigators found no difference in *FLT3* mutation rate between subjects with vs. without CNA. Since both of these studies were relatively small, follow up with larger cohorts will be required.

Although AML with mutation of genes encoding core binding factors (t(8;21) or inv(16)) indicates a relatively favorable prognosis, some of these subjects also do more poorly than expected. Akagi et al. used high density SNP arrays to study 48 newly diagnosed AML subjects with t(8;21) as the only abnormality identified by conventional cytogenetics (13). They found no CNA in 67% (Group A) and CNA in 33% (Group B). Since *KIT* mutation is associated with poor prognosis in t(8;21) AML, sequence analysis was performed. The authors identified activating *KIT* mutations in both groups, but concurrent amplification and mutation in Group B.

Correlation between Copy Number Alterations and Prognosis in MDS and AML

The significance of identifying CNA in AML would be increased by determining a correlation with prognosis. At present, most studies involve relatively small numbers of subjects and most of the recurrent gains or losses are individually present at low frequency. However, Walter et al. (9) found that CNA was more common in M6 and M7 AML in comparison to normal karyotype AML, suggesting correlation of CNA with relatively adverse prognosis. They also found that patients with 17q or 21q deletion had worse overall survival. However, the total number of CNA per patient did not correlate with survival, suggesting specific gains or losses might be prognosis driving. Karnan et al. found that CNA did not worsen remission rates or outcome in APL (12), but Agaki et al. (13) found CNA worsened prognosis in t(8;21) AML. The latter result is complicated by the fact that *KIT* mutations were slightly more common in the group with CNA.

Gohring et al. used array based CGH to study pediatric subjects with various congenital bone marrow failure disorders (14). They found correlation between CNA and progression to MDS/AML. These results suggest the possible role of screening such patients for CNA to predict progression and determine the timing of interventions such as stem cell transplant.

Copy Number Neutral Loss of Heterozygosity in MDS and AML

The use of SNP-microarray technology to identify CNA led to the realization that acquired CNN-LOH (or aUPD) is a frequent in AML. Raghavan et al. studied 64 subjects with newly diagnosed AML using a 10K SNP-microarray and identified CNN-LOH in 20% of all subjects (15). These studies identified a recurrent regions of CNN-LOH in AML on chromosomes 11p, 11q, 6p and 9q. These authors also investigated CNN-LOH at relapse in AML (16). They found aUPD in 40% of AML samples at relapse with the most common abnormality on 13q.

Similar to CNA, CNN-LOH is also common in normal karyotype AML. Akagi et al found that 32% of subjects with normal karyotype by G-banding cytogenetics exhibited CNN-LOH (8). The median size of the areas of LOH in this study was ~31 Mb. Recurrent regions of CNN-LOH included 1p, 6p, 8q, 13q and 19p. Walter et al also analyzed subjects with normal karyotype AML for CNN-LOH (9). Comparing leukemia samples to normal tissue for 34 subjects, they found CNN-LOH in only 15%. Additional studies with larger cohorts will be required to further clarify the frequency and significance of aUPD in normal karyotype AML. Since aUPD requires a recombination event, association between CNN-LOH and microsatellite instability was investigated by Serrano et al (17). In this study, 22 samples from subjects with normal karyotype AML were analyzed. Of the ~23% with CNN-LOH, 60% exhibited microsatellite instability in comparison to only 17% without CNN-LOH.

Studies of CNN-LOH in subjects with t(15;17) APL were also performed by Akagi et al (11). They found aUPD in 15% of all the APL subjects, including recurrent CNN-LOH involving 10q, 11p and 19q. However, this group found CNN-LOH in only 8% of subjects with newly diagnosed t(8;21) AML (13). Regions involved in aUPD included 11p, 11q and 6p. These studies identify 6p, 13q and 11p as common, recurrent regions of CNN-LOH in AML across multiple studies. This includes forms of AML with both favorable and unfavorable prognosis.

Correlation between Copy Number Neutral Loss of Heterozygosity and Mutation

In most forms of cancer, LOH is generally associated with loss of a copy of a tumor suppressor gene. Because some of the recurrent chromosomal regions of CNN-LOH in AML include genes that are involved in leukemia associated mutations, investigations were performed to determine if the leukemic clone is homozygous for the mutation. This would suggest that two copies of the mutant allele resulted in a competitive advantage and outgrowth of the clone. Fitzgibbon et al. studied 13 subjects with AML and CNN-LOH for the presence of two alleles of leukemia-associated mutations of *WT1* (11p), *FLT3* (13q), *CEBPA* (19q) and *RUNX1* (21q) (18). These genes are found at recurrent sites of CNN-LOH in AML. These investigators documented homozygous mutation of *FLT3* (4 of 4), *WT1* (1 of 3) and *RUNX1* (1 of 1). Raghavan et al. found homozygosity for internal tandem duplication (ITD) *FLT3* in AML samples at relapse (16). CNN-LOH with homozygous ITD-*FLT3* was also found in studies of normal karyotype AML (8,9), although this association was not found in a study of APL (11). Raghavan et al. found CNN-LOH of 19q was associated with two copies of a mutant form of *CEBPA* in a group of AML subjects at diagnosis (16). Additional studies will be required to determine the statistical significance of these associations between CNN-LOH and homozygous mutation of leukemia associated genes and the impact of this concurrence on prognosis.

Single Nucleotide Variants in MDS and AML

The next logical level of analysis of AML genomes would be identification of acquired single nucleotide variants (SNV). Since parallel sequencing of genomic DNA from the leukemia clone and unaffected tissues is required, this is a very labor intensive endeavor even with automated sequencing technology. One such study was recently reported by Mardis et al in which the authors sequenced 98% of the genome from a leukemic clone and normal skin of a subject with M1-AML (7). Twelve acquired somatic mutation were identified in coding sequences of genes and 52 in conserved or regulatory genomic regions. Targeted sequencing identified 4 of these 64 mutations in at least one sample from 188 additional AML subjects. This included mutation in *NRAS* and *NPM1* (previously described), and *IDH1* and a nongenic mutation in a conserved sequence (not previously described). These studies indicate both the power and difficulties with this approach.

Conclusion

Incorporation of classical G-banding cytogenetic analysis has altered prognostic assessment in AML. Technological advances permit a more detailed analysis of genomic losses or gains, and copy number neutral loss of heterozygosity. Combining mutation analysis with CNN-LOH studies may be particularly informative for prognostic considerations. Initial studies using these techniques have identified a number of recurrent chromosomal regions involved in CNA and CNN-LOH. Very early studies with massive parallel genomic sequencing indicate that recurrent SNV may also be identified using this approach.

At present, assessment of CNA or CNN-LOH is can be considered of potential importance to identifying additional prognostic indicators in AML. Further studies on larger populations will be required to determine the significance of specific abnormalities for prognosis or pathogenesis of AML. Of particular interest will be investigations to determine if aUPD with two copies of a gene containing a leukemia associated mutation worsens the prognosis in comparison to heterozygosity for the mutation. Genomic sequencing at this point is a research tool with further technical developments necessary to accumulate population data and assess the usefulness of identifying large numbers of leukemia associated SNV.

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