The Molecular Landscape of AML

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New Frontiers in Acute Myeloid Leukemia (AML) – Section 13

The Molecular Landscape of AML

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Take-home messages:

- There are at least 50 genes in which mutations have been identified in AML and which can co-occur to influence prognosis
- The frequency distribution of the mutations alters across the ages from infant, childhood, young and older adults
- Clonal evolution within patients will be a challenge for future therapeutic options or monitoring

Introduction

In recent years, technologies have advanced rapidly, moving away from the traditional cytogenetic analysis and beyond Sanger sequencing of candidate genes to whole genome or exome sequencing and the use of targeted mutation sequencing panels. The increase in capability and capacity with reducing costs and reduced turn-around times have significantly improved our knowledge of the diverse genomic landscape of AML. Many of the abnormalities have independent prognostic impact, although the majority co-occur with many other driver mutations to add to the complexity already associated with developing effective therapeutic strategies.

Current state of the art

Prior to 2008, abnormalities associated with AML had mainly been identified through cytogenetic analysis detecting mainly balanced reciprocal translocations. Those such as t(8;21)(q22;q22.1) (*RUNX1-RUNX1T1* gene fusion), inv(16)(p13.1q22) or t(16;16)(p13.1;q22) resulting in *CBFB-MYH11* fusion, are in favorable risk groups and the *KMT2A (MLL)* / 11q23 loci chromosomal abnormalities are in intermediate risk categories. Other recurring cytogenetic abnormalities are incorporated into the World Health Organization (WHO) or the European Leukemia Network (ELN) classifications.[1,2] Inv(3) (q21.3q26.2) or t(3;3)(q21.3;q26.2) leads to haploinsufficiency of *GATA2* and over-expression of *MECOM (EVI1)* and t(1;22)(p13.3;q13.1) resulting in the *RBM15-MKL1* fusion with involvement across chromatin organization, *HOX*-induced differentiation, and signaling pathways.[3] Others including t(9;22)(q34;q11), t(6;9)

(p23;q34); t(8;13)(p11;p13); t(7;12)(q36;p13) and those associated with *NUP98* fusions or 11p15 rearrangements are associated with extremely poor outcome. (Fig. 1).

A few gene mutations, such as those occurring in *FLT3*, *NPM1*, and *CEPBA* were incorporated into the WHO classification. Next-generation sequencing of a single AML patient in 2008[*4] and then subsequent studies involving larger cohorts of patient samples have demonstrated that mutations can occur in up to 50 genes in different functional categories.[5,*6,*7,8] Furthermore, almost all samples had mutations in more than one gene/functional group; some of these co-occurrences behave as positive or negative prognostic modifiers[7,8] and impact on a patient's response to treatment.[8]

The most frequent recurrent genetic abnormalities in AML can be coalesced into six main functional categories; the presence of multiple mutations in a single functional category is usually mutually exclusive however, it is possible to have mutations in different functional categories within a patient. Approximately 2/3rd of AML cases have mutations in genes leading to aberrant activation of signalling pathways; genes in this group include *FLT3*, *KRAS*, *NRAS*, *KIT*, *PTPN11*, *NF1*, *JAK2*, *CALR*, *CSF3R*, *CBL*, or *SETBP1*. *FLT3* mutations occur in approximately 1/3rd of patients, often in combination with *NPM1* or *DNMT3A* mutations. The second functional group are epigenetic modifiers, which have a role in DNA methylation and/or chromatin modification; these are *DNMT3A*, *IDH1*, *IDH2*, *TET2*, *ASXL1*, *EZH2*, or *KMT2A (MLL)*. Within this group, *DNMT3A*, a DNA methyltransferase, is the most commonly mutated gene occurring in 20% to 30% of cytogenetic standard risk AML. Mutations of the Nucleophosmin gene (NPM1) represent a functional group; present in around 32% of cases but, exhibits considerable molecular complexity through co-occurrence with other mutations, notably *DNMT3A* or *FLT3*-ITD, which can alter the prognostic significance.[9] Mutations in genes involved in RNA regulation/Spliceosome complex (*SRSF2*, *U2AF1*, *SF3B1*, *or ZRSR2*) occur in around 10% of patients and cause aberrant splicing as well as affecting the transcriptome and proteome. Around 23% of patients will have a mutation in a transcription factor, mainly in the myeloid transcription factors, such as *RUNX1* or *CEBPA* but this group also includes *GATA2*, *TP53*, *ETV6*, *BCOR*, *WT1*, and *PHF6*. The last functional category
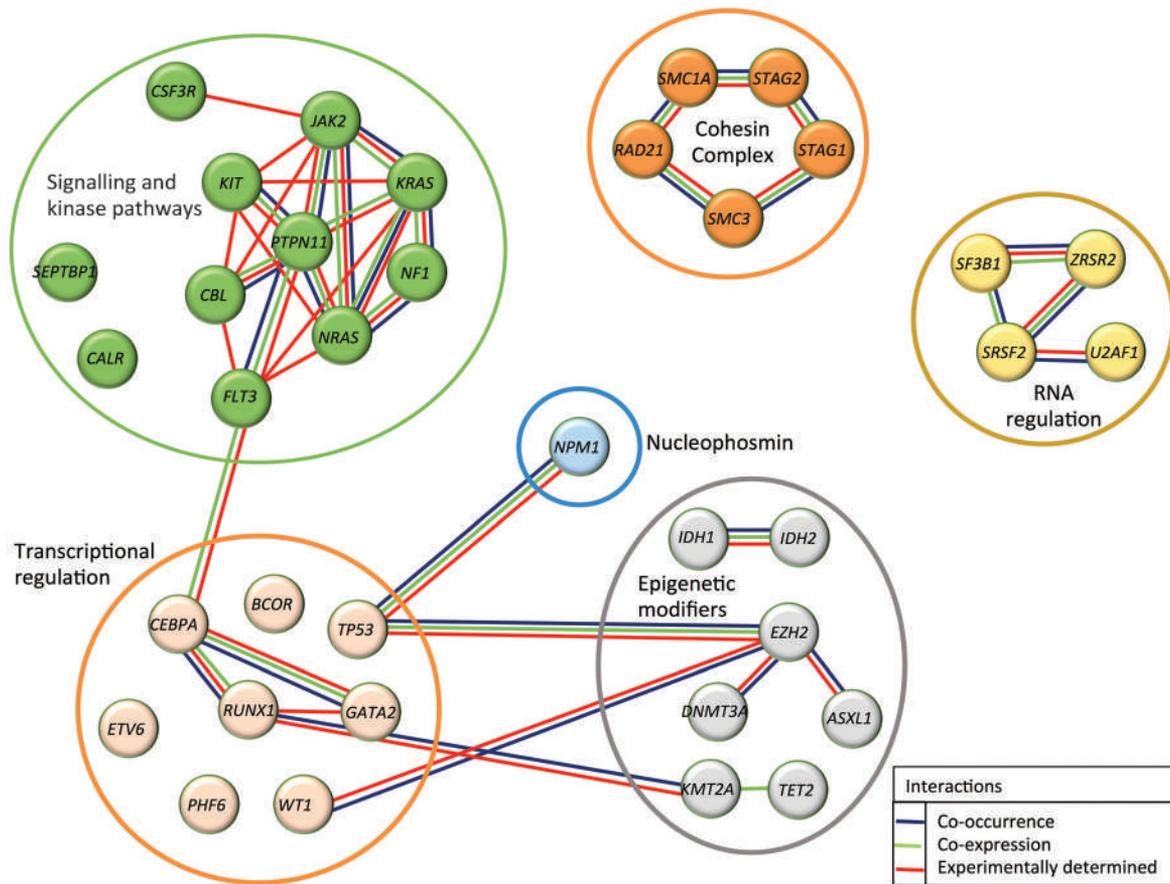
Figure 1. Protein–Protein Interaction Network of the commonly mutated genes in AML identified via STRING analysis (www.string-db. org).[22] Interactions shown are as experimentally determined (red lines), co-expression (green lines) or co-occurrence (dark blue lines). At this level the clustering of protein-protein interactions are limited mainly to the different functional groups; further supporting the mutual exclusivity of gene mutations within each functional group.

involves genes within the Cohesin complex (*RAD21, STAG1, STAG2, SMC1A,* or S*MC3*), which catalyses the folding of the genome into transcription associated loops. Although these mutations are less common (<10%) they usually co-occur with mutations from other functional classes. These mutations will also have an overall impact on the transcriptional landscape of the cells. Coding and non-coding, including lncRNA, miRNA and circRNA will be effected and have an impact on prognosis and as potential therapeutic targets.[10–12]

Gene mutations have been reported in AML patients across all age groups from infants to older adults; however, there is now increasing evidence to suggest that the spectrum of mutations is dynamic and changes with age. Germline mutations have been identified for some of these genes, notably GATA2 and CEBPA,[13] leading to the inclusion of "germline predisposition" as a separate WHO category[2] whilst the ELN has recommended molecular testing if this is suspected.[1] Whole genome sequencing of AML patients may also have the unintended consequences of identifying genes associated with altered therapeutic responses or the development of secondary cancers.

The majority of genomic studies are usually on younger adults, from 15 to 65 years old at diagnosis, and the incidence figures given above reflect this population age. However, there are clear age related differences in the mutational landscape of paediatric and adult AML.[*14] Pediatric AML has been shown to be more commonly associated with fusion oncogenes arising from chromosomal translocation, frequently involving the *KMT2A*

(*MLL*) gene. In addition, in infants and children, gene mutations often hit signaling and kinase pathway components, whereas mutations in adults more often occur in epigenetic regulators or transcription factors.[15] Furthermore, in a study of AML patients under 18 years old at diagnosis,[16]*NPM1* mutations were identified in only ~9% of childhood cases whilst *NRAS* mutations were detected in 30% of pediatric AML (c.f. 32% and ~10% respectively in adult AML).[8] Indeed, recurrent focal deletions are more common in pediatric cases specifically involving ZEB2, MBNL1, and ELF1.[*14]

At the other end of the age spectrum, in patients over the age of 60, the top 5 mutated genes with an occurrence above 20% were *NPM1, DNMT3, FLT3-ITD, TET2,* and *SFSF2*.[17] A shift in genes and functional groups mutated in different age groups is demonstrated by mutations in genes associated with epigenetic regulation, notably DNA methylation, observed in 73% of patients over the age of 80 years old at diagnosis.[18] The occurrence of gene mutations in patients with clonal hematopoiesis of indeterminate potential (CHIP) will not be discussed except to highlight that *DNMT3A, TET2,* and *ASXL1* (epigenetic modifiers) are frequently mutated in this aging patient population; occurring during the natural ageing process and pathogenicity cannot be directly correlated to these mutations.[19]

In addition to the changes in the mutational landscape due to age, the spectrum of mutations within individual patients throughout AML progression is not static. While the variability was limited to sub-clones with late-acquired mutations, evolution

mainly involved modification of sub-clones leaving the clonal background unchanged.[20] However, single cell analysis has demonstrated further genetic complexity with a preferred order of mutation acquisition and simultaneous sub-clone evolution.*[21]

Future perspectives

The challenges that lie ahead are not associated with identifying more mutated genes, although rare infrequent mutations may be identified with increasing numbers of patients sequenced. The challenge is how the global architecture of the disease functions through interactions between mutations and how their co-occurrences influences therapeutic options. Thus, consideration must be given to how we approach and treat the complex nature of the disease as a whole as opposed to targeting single gene mutations.

References

1. Dohner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood*. 2017;129:424–447.
2. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*. 2016;127:2391–2405.
3. Kayser S, Levis MJ. Clinical implications of molecular markers in acute myeloid leukemia. *Eur J Haematol*. 2019;102:20–35.
*4. Ley TJ, Mardis ER, Ding L, et al. DNA sequencing of a cytogenetically normal acute myeloid leukaemia genome. *Nature*. 2008;456:66–72.

This paper reported the first DNA sequencing of an AML patient and established whole-genome sequencing as a method for discovering cancer-initiating mutations in previously unidentified genes.

5. Mardis ER, Ding L, Dooling DJ, et al. Recurring mutations found by sequencing an acute myeloid leukemia genome. *N Engl J Med*. 2009;361:1058–1066.
*6. Ley TJ, Miller C, et al. Cancer Genome Atlas Research NetworkGenomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N Engl J Med*. 2013;368:2059–2074.

Showed the variation of mutational profiles across 200 AML patients and which genes could co-occur.

*7. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic classification and prognosis in acute myeloid leukemia. *N Engl J Med*. 2016;374:2209–2221.

Showed the driver landscape in AML with molecular subgroups that inform disease classification and prognostic stratification.

8. Tyner JW, Tognon CE, Bottomly D, et al. Functional genomic landscape of acute myeloid leukaemia. *Nature*. 2018;562:526–531.
9. Grimwade D, Ivey A, Huntly BJ. Molecular landscape of acute myeloid leukemia in younger adults and its clinical relevance. *Blood*. 2016;127:29–41.
10. Papaioannou D, Volinia S, Nicolet D, et al. Clinical and functional significance of circular RNAs in cytogenetically normal AML. *Blood Adv*. 2020;4:239–251.
11. Gourvest M, Brousset P, Bousquet M. Long noncoding RNAs in acute myeloid leukemia: functional characterization and clinical relevance. *Cancers (Basel)*. 2019;11. pii: E1638. doi: 10.3390/cancers11111638.
12. Handschuh L. Not only mutations matter: molecular picture of acute myeloid leukemia emerging from transcriptome studies. *J Oncol*. 2019;2019:7239206.
13. Charrot S, Armes H, Rio-Machin A, et al. AML through the prism of molecular genetics. *Br J Haematol*. 2020;188: 49–62.
*14. Bolouri H, Farrar JE, Triche TJr, et al. The molecular landscape of pediatric acute myeloid leukemia reveals recurrent structural alterations and age-specific mutational interactions. *Nat Med*. 2018;24:103–112.

Demonstrated that paediatric AML patients have a distinct and different mutational landscape than adult AML patients. They highlighted the need for the development of age-tailored targeted therapies.

15. Mercher T, Schwaller J. Pediatric acute myeloid leukemia (AML): from genes to models toward targeted therapeutic intervention. *Front Pediatr*. 2019;7:401.
16. Marceau-Renaut A, Duployez N, Ducourneau B, et al. Molecular profiling defines distinct prognostic subgroups in childhood AML: a report from the French ELAM02 study group. *HemaSphere*. 2018;2: e31.
17. Eisfeld A-K, Kohlschmidt J, Mrózek K, et al. Mutation patterns identify adult patients with de novo acute myeloid leukemia aged 60 years or older who respond favorably to standard chemotherapy: an analysis of Alliance studies. *Leukemia*. 2018; 32:1338–1348.
18. Renaud L, Nibourel O, Marceau-Renaut A, et al. Comprehensive molecular landscape in patients older than 80 years old diagnosed with acute myeloid leukemia: A study of the French Hauts-de-France AML observatory. *Am J Hematol*. 2019;94:E24–E27.
19. Link DC, Walter MJ. 'CHIP'ping away at clonal hematopoiesis. *Leukemia*. 2016;30:1633–1635.
20. Herudkova Z, Culen M, Folta A, et al. Clonal hierarchy of main molecular lesions in acute myeloid leukaemia. *Br J Haematol*. 2019 Dec 10. doi: 10.1111/bjh.16341. [Epub ahead of print].
21. Potter N, Miraki-Moud F, Ermini L, et al. Single cell analysis of clonal architecture in acute myeloid leukaemia. *Leukemia*. 2019;33:1113–1123.

Provided evidence, at the single cell level, for genetic variation in acute leukaemia and that a preferential order of mutation accrual and parallel evolution of sub-clones occurs.

22. Szklarczyk D, Gable AL, Lyon D, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res*. 2019;47:D607–D613.