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Aplastic anemia in adult and pediatric hematology - Section 2

Somatic mutations in aplastic anemia: significance for classification, therapy, and outcome

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Take home messages

- Somatic mutations occur in 70% of AA patients, and up to a third have myeloid-specific mutations.
- The immune response to somatic mutations contributes to determining their fate and impact in AA.
- The full significance of myeloid-specific somatic mutations in AA requires correlation with cytomorphological and cytogenetic features and future serial sampling in prospective clinical studies.

Introduction

Next generation sequencing has enabled detection of clonal hemopoiesis in many more patients with aplastic anemia (AA) than previously realized by conventional metaphase cytogenetics, FISH or whole genome scanning using SNP-karyotyping and flow cytometric detection of PNH clones. An abnormal clone can expand in an aplastic BM through selection exerted by an autoimmune attack, for example, where there is loss of certain HLA class I alleles, through loss of heterozygosity (LOH) for 6p, or by structural somatic HLA allelic mutations,[1,2] or with clonal expansion of GPI-deficient PNH clones that escape the autoimmune attack,[*3] and/or somatic mutations (SM) can also emerge randomly in AA through genetic drift, whereby a mutant clone can more easily expand in a hypocellular bone marrow.[*3,4] Some SM may be preleukemic, but others may be neutral or even beneficial.

Current state of the art

Impact of the immune signature of AA and MDS on somatic mutations

The immune signature of AA is defined by a proinflammatory environment with combined expansion of T helper (Th)1 (clonal) and Th17 cells, and a reduction in T regulatory cells (Tregs) that are dysfunctional in terms of their ability to suppress autoreactive cytotoxic CD8 T cells.[*5,6] In low-risk MDS Tregs are normal but Th17 cells are increased. In high risk MDS, increased Tregs and myeloid-derived suppressor cells and features of smoldering/chronic inflammation, result in a switch from immune surveillance to immune-subversion and subsequent disease progression (Fig. 1).[7,8,*9] The immune response (innate and adaptive) likely plays a key role in modulating the fate of abnormal mutated clones in AA, but other factors include the cellular origin and type of mutation, clone size, neoantigen formation,[10] ethnicity and age, and possible defective DNA repair mechanisms.

SM that arise through immune escape

PNH clones are detected in up to 50% of AA patients using flow cytometry, which is more sensitive than PIGA sequencing for a clone size of <10%.[*11,*12] SM in PIGA are predictive of response to immunosuppressive therapy (IST), and good prognosis. 6pLOH is relatively specific to AA and occurs in up to 19% of patients, compared to 1% in MDS and is very rare in the general population. 6pLOH favors loss of specific HLA alleles such as HLA-B*40:02, B*54:01. SM in HLA-B40:02 leading to loss of function phenotype have also been detected in patients who show high response to IST and low risk of progression to MDS/AML.[1,2]

Myeloid-specific SM in AA

Approximately one third of AA patients have a myeloid-specific SM, but they occur less frequently than in MDS.[*11,*12,13–15] Differences in frequencies reflect differences in methodologies, depth of sequencing, age, and stage of AA. Genes mutated commonly involve *ASXL1*, *DNMT3A*, or *BCOR/BCORL1*, and there is underrepresentation of *TET2*, *JAK2*, *RUNX1*, and *TP53*. ASXL1 and DNMT3A clones do more often expand over time in AA, but not in all cases. Some of the myeloid-specific SM are the same ones that are seen in ARCH, and in AA their incidence increases with age.[16] Other differences in SM between AA, MDS and AML are summarized in Fig. 1. In contrast to *PIGA*, and *BCOR/BCORL1*, patients with *DNMT3A*, *ASXL1*, *TP53*, *RUNX1*, or *CSMD1* treated with IST, show worse response, overall and progression free survival.[*12] Presence of SM after IST

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	ARCH [17]	AA [12-14]	Hypoplastic MDS [18]	MDS [19,20]
Frequency of SM	9.5% for age 70-79yr 11.7% for age 80-89yr	19% - 45%	38%	70-80%
The most common SM	DNMT3A, TET2, ASXL1, JAK2	ASXL1, DNMT3A, BCOR/BCORL1, DNMT3A, ASXL1	Lower prevalence of splicing factor SMs, ASXL1 and TET2 compared to MDS, and higher prevalence than AA	SF3B1, TET2, ASXL1, DNMT3A, SRSF2, RUNX1
Prognosis	Increased risk of all-cause mortality, haematological cancer, coronary heart disease, ischemic stroke, Diabetes type 2	Good prognosis: BCOR/BCORL1; poor prognosis: ASXL1, DNMT3A [13] For all SMs together, 38% risk of later MDS/AML [12]	Integrated morphology + genetic (chromosome +SM) score distinguishes cases into (1) non-malignant BMF with low risk AML and (2) true hypoplastic MDS	Good prognosis: SF3B1 Poor/neutral: the rest
Mean VAF%	9 - 19%	9-26% (<10% in 40% of patients)	38%	30 -42%
Mutations per patient	1 (in 93% individuals)	1 (in 64-90% patients)	Median 1 (1-4)	Median 3 (0-12)

Figure 1. The pattern of somatic mutations (SM) in aplastic anemia, hypoplastic myelodysplastic syndrome (MDS) and MDS.

is associated with increased risk of MDS/AML, especially for ASXL1, RUNX1 and splicing factor SM with high VAF%, higher number of SM per patient and longer duration of AA.[*11] In contrast, recent studies failed to show an associated risk of MDS with DNMT3A and TET2.[13]

SM in AA compared to hypoplastic MDS

The distinction between AA and hypoplastic MDS is challenging morphologically. In a recent collaborative King's College London/University of Pavia study, a new diagnostic score discriminated between 278 patients with hypoplastic MDS and 136 with AA.[17] In hypoplastic MDS the prevalence of SM, their VAF and number of SMs per patient, were intermediate between the patterns seen in AA and normo-/hypercellular MDS (see Fig. 1). Incorporating SM with morphological data enabled separation of hypoplastic MDS patients into 2 groups, one with clinical and genetic features highly consistent with myeloid neoplasm and one with features of non-malignant bone marrow and absence of progression to AML.

Future perspectives

Following IST for AA, late clonal progression to MDS/AML increases to a maximum of 15% to 26% at 10 years.[15] 19% of patients with refractory SAA treated with eltrombopag, developed abnormal cytogenetic clones (mostly abnormalities of chromosome 7) at a median of 3 months.[18] Key to the understanding the significance of SM in AA is serial analysis over time, particularly in a disease where abnormal cytogenetic clones over time may evolve, remain stable, or even disappear. This, and the possible contribution of eltrombopag to later clonal progression in the context of IST, will be addressed in the prospective randomized study, EBMT RACE trial of first line horse ATG, ciclosporin with or without eltrombopag (ClinicalTrials.gov, NCT02099747) currently in progress. Serial samples for SM, and high dimensional immune-phenotyping will examine the immune response to SM.[6] Currently the finding of a myeloid-specific SM in AA in the absence of morphological features of MDS or an MDS-defining cytogenetic abnormality, should not trigger a change in treatment, but the full blood count and SM should be monitored carefully along with an early assessment of potential hemopoietic stem cell transplant donor availability, in the event of subsequent early disease progression[19–20].

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