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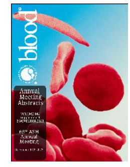

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

508.BONE MARROW FAILURE: ACQUIRED

Single-Cell Dissection Reveals a Distinct Origin of Small Paroxysmal Nocturnal Hemoglobinuria Clones in Immune Aplastic Anemia and Healthy Individuals

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Abstract

Introduction

Paroxysmal nocturnal hemoglobinuria (PNH) clones are characterized by the absence of cell surface glycosylphosphatidylinositol (GPI)-anchors owing to somatic mutations in the *PIGA* gene on the X chromosome. Large PNH clones derived from *PIGA* mutant multipotent progenitors (MPPs) result in clinically significant hemolysis. In immune aplastic anemia (AA), the PNH clone represents immune escape from T cell-mediated pathogenesis and is usually small (<1%), sometimes likely derived from lineage-committed progenitors (LCPs), with detectable GPI-anchor deficient (PNH-type) cell populations only in granulocytes and monocytes. Small PNH-type cells are also present in healthy individuals at a much lower frequency (<0.003%) than in AA patients. The nature of the small PNH-type cells is not fully understood because of the difficulties in analyzing rare cell populations.

Methods

In this study, we developed a method to detect *PIGA* mutations within a small population of cells at single-cell resolution. PNH-type cells were enriched from peripheral blood leukocytes using magnetic microbeads and monoclonal antibodies specific for GPI-anchored proteins. The enriched cell fraction was then subjected to fluorescence-activated cell sorting after staining with lineage-specific markers and fluorescein-labelled proaerolysin. We sorted a limited number (100 cells in males and 50 cells in females) of PNH-type granulocytes (PNH-Gs) and T cells (PNH-Ts) as well as wild-type cells into separate tubes, and their *PIGA* genes were directly amplified by multiplex PCR without a DNA extraction procedure for subsequent deep nucleotide sequencing. To ensure the specificity of *PIGA* mutation detection, we set cut-off thresholds of 2% for single-nucleotide substitutions and 1% for indels, in addition to the thresholds based on base-position-specific error rates in 10 sets of 2000 wild-type cells, as previously reported (Zaimoku et al. Blood 2021).

Results

In 19 sets of 50-100 wild-type cells from 13 individuals, we detected only 7 mutations with variant allele frequencies (VAFs) ranging from 2.1% to 4.2% (Figure 1). Three of these were synonymous mutations, and the remaining 4 were missense mutations, suggesting that these mutations may be true passenger mutations rather than sequencing errors. In all 6 healthy individuals, small PNH-type cells exhibited a polyclonal pattern. We detected numerous small, likely inactivating, *PIGA* mutations in PNH-Gs, with a median of 11 (range, 6-18) mutations per 100 cells. In 3 individuals assessed twice, approximately half of the *PIGA* mutations were detected in both sets of PNH-Gs, whereas the remaining mutations were unique to each set. Similarly, PNH-Ts from 4 healthy individuals showed polyclonality, with a median of 14 (range, 6-22) mutations per 100 cells. Interestingly, *PIGA* mutations were not shared between PNH-Gs and PNH-Ts, except for 2 splice site mutations with VAFs <10%. The polyclonal pattern was further confirmed by Sanger sequencing, which successfully detected 7 unique *PIGA* mutations in 5 sets of 5 PNH-type cells (instead of 100 cells) in a healthy donor, whereas no mutations were detected in 3 sets of 5 wild-type cells, except for 1 synonymous mutation. In contrast, small PNH-Gs of 5 AA patients, with frequencies of 0.02%, 0.08%, 0.09%, 0.2%, and 0.5% of total granulocytes, were primarily derived from single *PIGA* mutant clones, which

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accounted for 90%-99% of PNH-G populations. In another patient with AA, PNH-Gs with a frequency of 3.3% were derived from 2 dominant clones. Notably, the dominant *PIGA* mutations in the PNH-Gs of AA patients were all detectable in PNH-Ts, even in 2 patients whose PNH-Ts were not detectable (<0.003%) and in 3 patients whose percentages of PNH-Ts were 0.004%, 0.02%, and 0.08% before microbead-based enrichment.

Discussion

Our novel sequencing approach provides insight into the clonality and lineage distribution of small PNH-type cells without *in vitro* culture manipulation. In healthy individuals, most PNH clones originate from LCPs, where many cell divisions occur, thereby providing opportunities for the acquisition of *PIGA* mutations. In immune AA, only *PIGA* mutations acquired at the MPP level lead to clonal proliferation, strongly suggesting that the immune pathogenesis of AA targets MPPs, rather than LCPs. A clonality analysis of small PNH-type cells thus helps diagnose the immune pathogenesis of AA.

Disclosures Zaimoku: *Nippon Shinyaku:* Research Funding. **Yamazaki:** *Kyowa Kirin:* Honoraria; *Novartis Pharma:* Honoraria. **Miyamoto:** *Otsuka Pharmaceutical:* Honoraria; *MSD:* Honoraria; *Daiichi Sankyo:* Honoraria; *Novartis:* Honoraria; *Bristol:* Honoraria; *Janssen:* Honoraria; *Astellas:* Honoraria; *Kyowa Kirin:* Honoraria, Research Funding; *AbbVie:* Honoraria; *Amgen:* Honoraria; *Chugai Pharmaceutical:* Research Funding. **Nakao:** *Novartis Pharma:* Honoraria; *Kyowa Kirin:* Honoraria.

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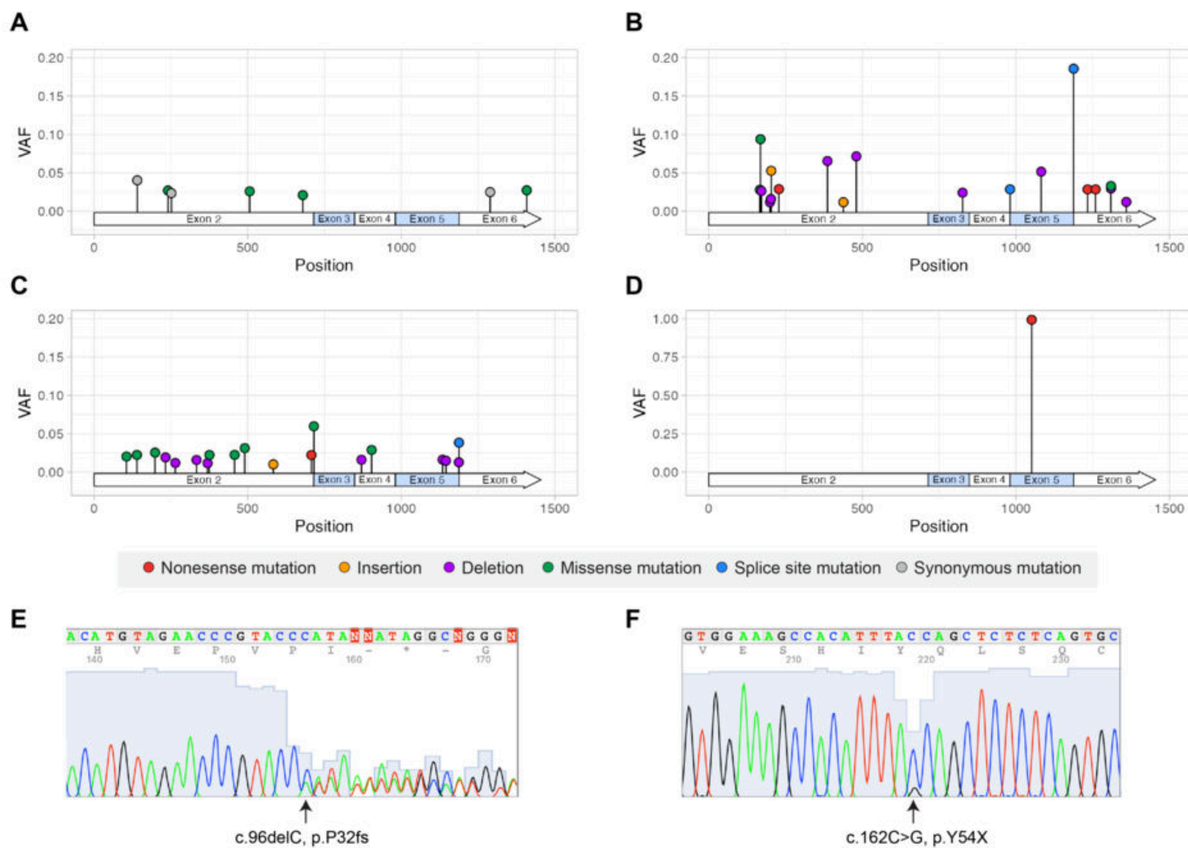

Figure 1. Somatic *PIGA* mutations in small cell populations. (A) *PIGA* mutations found in 19 sets of 50-100 wild-type cells from 13 individuals. (B) *PIGA* mutations in a single set of 100 PNH-Gs from a healthy male individual, undetectable before enrichment. (C) *PIGA* mutations in a single set of 100 PNH-Ts from a healthy male individual, undetectable before enrichment. (D) *PIGA* mutations in a single set of 100 PNH-Gs from a male AA patient who had 0.09% of PNH-Gs in total granulocytes. (E) A frameshift deletion (c.97delC) in 5 PNH-Gs of a healthy male individual detected by Sanger sequencing. (F) A nonsense mutation (c.162C>G, p.Y54X) in 5 PNH-Ts of a healthy male individual detected by Sanger sequencing.

Figure 1

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