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LEUKAEMIA

A pre-leukaemic reservoir

> pre-leukaemic HSCs have a competitive advantage over normal HSCs

Early events in the development of leukaemias are not well defined. For example, it is unclear whether cells that are non-tumorigenic but contain clinically relevant alterations exist and, if so, whether these cells remain present through the course of the disease.

While using deep targeted sequencing of genes that are known to be mutated in leukaemia to analyse intratumour heterogeneity in patients with acute myeloid leukaemia (AML), John Dick and colleagues discovered that not only leukaemic samples but also normal T cells from the same patients carried mutations in DNA (cytosine-5-)-methyltransferase 3 alpha (*DNMT3A*^{mut}). Mutation of another known AML gene, nucleophosmin (*NPM1*), and the *FLT3* internal tandem duplication (*FLT3*-ITD), often co-occur with *DNMT3A*^{mut}. Although coincident *NPM1* mutations (*NPM1c*) and *FLT3*-ITD were present at similar allele frequencies to *DNMT3A*^{mut} in leukaemic blasts, they did not co-occur in T cells, which indicates that *DNMT3A*^{mut} arises early and occurs in cells that can give rise to T cells. Examination of several non-leukaemic, haematopoietic cell populations showed that *DNMT3A*^{mut} occurs without *NPM1c* in many lineages, including haematopoietic stem cells/multipotent progenitors (HSCs/MPPs). In HSCs/MPPs, the allele frequency of *DNMT3A*^{mut} was substantially higher than estimated for the clonal contribution from a normal HSC, which suggests that clonal expansion of these cells occurs.

Analysis of samples from five patients with AML, which were taken at diagnosis, remission and relapse, indicated that many haematopoietic cell populations at remission and relapse contained *DNMT3A*^{mut} but not *NPM1c*, and in one patient with long-term remission, an increase in the *DNMT3A*^{mut} allele frequency was observed over time. This suggests that cells carrying *DNMT3A*^{mut} can survive chemotherapy and can expand, potentially creating a reservoir of cells that are primed for relapse. To determine whether cells with *DNMT3A*^{mut} can function as HSCs, xenograft repopulation assays in immunocompromised mice were carried out with HSCs/MPPs that were derived from two different patients (who had *DNMT3A*^{mut} allele frequencies of 30% and 20% in these cells). Many of the mice had multilineage engraftment, with a large proportion of cells of different lineages carrying *DNMT3A*^{mut} but not *NPM1c*, which indicates that the *DNMT3A*^{mut}-carrying cells are indeed functional HSCs. Furthermore, the allele frequency of *DNMT3A*^{mut} in the mice increased over time, which suggests that these pre-leukaemic HSCs have a competitive advantage over normal HSCs.

A similar situation might occur in leukaemias with mutations in isocitrate dehydrogenase 1 (*IDH1*) or *IDH2*. The authors looked back at previous xenograft repopulation data from 25 patient AML samples that had generated multilineage grafts. Of these, 10 were from patients with *DNMT3A*^{mut}, and 12 had *IDH1* or *IDH2* mutations. Analysis of purified HSC and progenitor populations from six further patients with *IDH1* or *IDH2* mutations indicated that two of these patients had mutant *IDH2* without *NPM1c*, which suggests that *IDH2* mutations might also generate pre-leukaemic cells.

Several interesting insights arise from these results. In addition to shedding light on the order of mutational events in AML and showing that pre-leukaemic HSCs are present at diagnosis, the data suggest that these cells are resistant to chemotherapy and could contribute to relapse. Therefore, the presence of these cells might need to be included in the definition of minimal residual disease, and the eradication of these pre-leukaemic HSCs might be necessary to prevent relapse.

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ORIGINAL RESEARCH PAPER Shlush, L. I. *et al.* Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. *Nature* **506**, 328–333 (2014)