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Clonal evolution of pre-leukemic hematopoietic stem cells precedes human acute myeloid leukemia

Ravindra Majeti, MD, PhD, Assistant Professor *

Department of Internal Medicine, Division of Hematology, Cancer Institute, and Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, USA

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Massively parallel DNA sequencing has uncovered recurrent mutations in many human cancers. In acute myeloid leukemia (AML), cancer genome/exome resequencing has identified numerous recurrently mutated genes with an average of 5 mutations in each case of de novo AML. In order for these multiple mutations to accumulate in a single lineage of cells, they are serially acquired in clones of self-renewing hematopoietic stem cells (HSC), termed pre-leukemic HSC. Isolation and characterization of pre-leukemic HSC have shown that their mutations are enriched in genes involved in regulating DNA methylation, chromatin modifications, and the cohesin complex. On the other hand, genes involved in regulating activated signaling are generally absent. Pre-leukemic HSC have been found to persist in clinical remission and may ultimately give rise to relapsed disease through the acquisition of novel mutations. Thus, pre-leukemic HSC may constitute a key cellular reservoir that must be eradicated for long-term cures.

Introduction

Acute myeloid leukemia (AML) is an aggressive malignancy of bone marrow progenitors that is associated with the clonal accumulation of immature myeloid blasts defective in their maturation and

* Lorry Lokey Stem Cell Research Building, 265 Campus Drive, G3021B, Stanford, CA 94305, USA. Tel.: +1 650 721 6376; Fax: +1 650 736 2961.
E-mail address: rmajeti@stanford.edu.

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function [1]. Patients generally come to medical attention with symptoms related to bone marrow failure: fatigue resulting from anemia, infection due to leukopenia, or bleeding due to thrombocytopenia. Current standard of care in medically fit patients involves high-dose chemotherapy and in many cases allogeneic hematopoietic cell transplantation. Both of these therapies result in significant morbidity and mortality, particularly in patients over the age of 60 years who constitute the majority of cases. Even with these aggressive therapies, 5-year overall survival is approximately 30%, and is much lower in elderly patients due to both refractory disease and inability to tolerate therapy. Strikingly, the standard of care in AML has not changed in decades, despite extensive mechanistic and clinical research [2].

Genomics

The advent of massively parallel next-generation DNA sequencing has been rapidly applied to the investigation of cancer genomes resulting in the identification of recurrently mutated genes in human cancer [3–5]. This is particularly true for human AML where several hundred patient samples have been analyzed using these methods. A landmark study from The Cancer Genome Atlas (TCGA) AML group reported the results of whole genome or whole exome sequencing of 200 cases of human AML [6]. These investigators identified the landscape of recurrently mutated, and therefore likely driver, genes including several novel genes that are now being intensely investigated. These genes were classified into 9 distinct groups based on known and/or predicted functions, and mutations within the same group tended to be mutually exclusive. In addition, this work has led to a broader understanding of mutational burden in AML where the TCGA reported that on average, each case of AML harbors 5 mutations in recurrently mutated genes. This observation raises several important questions: (1) How do these multiple mutations accumulate in a single lineage of cells? (2) Which mutations are the founder mutations, initiating the leukemogenic process? and (3) What is the nature and genomics of pre-leukemic cells in de novo AML?

Human hematopoiesis

In order to start addressing these questions, it is first necessary to consider normal human hematopoietic development. Human hematopoiesis is organized as a cellular hierarchy initiated and maintained by long-term self-renewing hematopoietic stem cells (HSC) [7]. HSC in turn give rise to multipotent progenitors, which produce lineage-restricted progenitors, and eventually all the mature, differentiated cells of the blood. In normal myeloid development, mature monocytes and granulocytes have short life spans measured in hours to days, while lineage-restricted and multipotent progenitors survive for only a few weeks [8].

The short life span of these cells links to the first question: How do multiple mutations accumulate in a single lineage of cells? We have hypothesized that since most cells in the myeloid differentiation hierarchy are short-lived, then leukemogenic mutations must serially accumulate in successive clones of self-renewing HSC [9,10]. According to this model, in AML patients, the rare residual HSC compartment will consist of some normal cells lacking all mutations, but will also contain some pre-leukemic HSC that contain some, but not all, of the mutations identified in the downstream leukemia. Moreover, our model proposes that these mutations will be serially acquired in clones of HSC. Thus, if the leukemia is associated with 5 mutations in genes 1–5, clones of pre-leukemic HSC contain mutation in gene 1 alone, genes 1 and 2, genes 1–3, and genes 1–4 should be present that will indicate the history of mutational acquisition in the individual AML.

We set out to investigate this model through the investigation of de novo AML. This study was facilitated by recent advances in the identification of surface markers that distinguish leukemia cells from normal hematopoietic stem cells [11,12]. Our overall approach was to: (1) purify residual HSC from AML samples taken at diagnosis using the novel cell surface markers in FACS; (2) genotype the individual leukemia by exome sequencing using purified patient-matched T cells as the reference; (3) design primers to amplify genomic regions harboring mutations and subject each to targeted deep resequencing to identify the presence and frequency of leukemia-specific mutations present in residual HSC – these would be pre-leukemic mutations and indicate the presence of pre-leukemic HSC; and (4)