IN THE SPOTLIGHT

iPSC Models of Leukemia Come of Age

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Summary: In this issue of *Blood Cancer Discovery*, Kotini and colleagues present a strategy for large-scale reprogramming of primary human acute myeloid leukemias (AML) to induced pluripotent stem cell (iPSC). They show that the hematopoietic differentiation of AML iPSCs gives rise to transplantable leukemias with remarkable molecular similarity to the original patients' AML, providing new models and insights into the disease.

See related article by Kotini et al., p. 318 (7).

Acute myeloid leukemia (AML) is a clonal disorder driven by the acquisition of somatic genetic lesions in hematopoietic stem cells (HSC; ref. 1). The discovery of clonal hematopoiesis has cemented the idea that leukemias evolve through an extended period of preleukemic clonal evolution (2). Capturing this process has been a challenge for experimental models of leukemia, especially human cell models. There has been a growing appreciation that the differences in human HSC biology and oncogene function motivate the need for human cell models of leukemia (3). In principle, leukemogenesis can be reconstituted by engineering mutations into normal HSCs. However, human cells are relatively refractory to transformation, and existing models are driven by retroviral expression of powerful oncogenes such as MLL fusions (4). On the other hand, improved humanized mouse models have enabled *in vivo* xenotransplantation of most human AML samples. However, genetic modification of primary AMLs is challenging and does not provide insights into preleukemic evolution. AMLs often harbor chromosomal abnormalities, which are difficult to engineer or model in mice. Reprogramming to pluripotency involves the delivery of OSKM transcription factors to convert single cells into induced pluripotent stem cells (iPSC; ref. 5). It has been known for a long time that cancer cells can be reprogrammed to pluripotency (6), though the process has been perceived as unreliable and biased in favor of specific mutations. In this issue, Kotini and colleagues present a large-scale strategy showing that primary AMLs can be efficiently reprogrammed to iPSCs (7). What is more, hematopoietic differentiation of AML iPSC gives rise to transplantable leukemias with remarkable molecular similarity to the original patients' AML.

Starting from a panel of 33 AML patient samples, Kotini and colleagues successfully reprogrammed 15 of them, generating a panel of 21 distinct genetic clones and 24 different driver lesions, and isogenic normal iPSCs derived from normal blood cells from many patients. This represents about 1 in 2 likelihood of successful reprogramming. As reprogramming is stochastic, multiple genetic subclones can be captured from an individual patient informing mutation order during clonal evolution (8). Indeed, several leukemic or preleukemic clones were reprogrammed in a number of patients in this study. Not all AML samples could be reprogrammed. AML with *NPM1* mutations were refractory to reprogramming, which may suggest that the NPM1 function is essential for the establishment of pluripotent state. Interestingly, 5q deletions encoding *NPM1* are also refractory to reprogramming. Although not all leukemias can be reprogrammed, this study dispels the notion that leukemias are difficult or impossible to reprogram. It would be interesting to see if other reprogrammed cancers, such as pancreatic adenocarcinoma (9), can also be efficiently reprogrammed at scale and give rise to tumors following differentiation.

Starting from this large panel of AML iPSCs, Kotini and colleagues performed hematopoietic differentiation to generate genetically accurate models of leukemia. Current protocols do not generate transplantable HSCs from normal iPSCs without the use of exogenous transcription factors. By contrast, Kotini and colleagues show that the hematopoietic differentiation of iPSCs derived from 6 patients with AML gives rise to lethal serially transplantable leukemias after xenotransplantation. Single-cell RNA sequencing analysis revealed a remarkable molecular similarity of primary and iPSC-derived AML cells, especially following xenotransplantation. Transplantation in both cases caused a depletion of leukemia stem cell (LSC)–like cells in favor of leukemic blasts with a myeloid profile, reflecting gradual limited differentiation of LSC into more mature blasts. Interestingly, analysis of a more advanced leukemic clone with the *FLT3*-ITD mutation and its precursor clone revealed increased self-renewal and a higher proportion of LSCs after transplantation. The ability to study multiple genetic leukemic or preleukemic subclones in parallel, which cannot be prospectively isolated from primary AML, represents an important advantage of the iPSC approach.

The potential of AML iPSC to reestablish leukemic hierarchy with a striking resemblance to the original AML raises interesting implications for our understanding of how genetic and epigenetic states contribute to leukemogenesis. A seminal study by Hochedlinger and colleagues showed

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that reprogramming of RAS-inducible melanoma by somatic cell nuclear transfer gives rise to chimeric mice that develop melanoma with high penetrance, arguing that the genetic mutation is sufficient to reestablish the epigenetic state of the original tumor (6). Although we consider leukemia to be a genetic disease, the epigenetic state is critical to disease progression, evidenced by the prevalence of mutations in key epigenetic modifiers in AML. Epigenetic marks, including DNA and histone methylation, are largely erased during reprogramming to pluripotency (10). The epigenetic state of AML blasts is presumably reset after reprogramming and then reestablished during hematopoietic differentiation of iPSC into AML. The authors did not formally assess how faithfully global DNA methylation or histone modifications are erased and then reestablished in iPSC-derived leukemias compared with the original AML. However, the implication is that given the cues from the xenograft environment, genetic mutations may be sufficient to reestablish a leukemic epigenetic state.

The large panel of genetically defined iPSC clones generated by Kotini and colleagues and our parallel collection of preleukemic iPSCs (8) are versatile disease models and important resources for the community. Does this mean that the field will be rushing to adopt the iPSC system? This is the hope, however, the hurdle remains high for individual labs to adopt these systems due to the practical challenges of working with iPSCs. These include mastering protocols for maintaining iPSCs, hematopoietic differentiation, and the high cost of specialized media and reagents used in these protocols. Instead of working with iPSCs directly, a practical option is to bank differentiated AML cells, which can be massively expanded *in vitro*. iPSC-derived preleukemic and MDS hematopoietic progenitors can also be expanded using conditional expression of transcription factors (8). This goal could be achieved by facilitating the adoption of these resources by experienced stem cell cores, now established in most large institutions, enabling easier distribution and wider adoption of these models by the community. As this technology becomes more accessible, we anticipate that the growing panel of AML patient iPSCs will provide important insights into disease biology and therapeutic strategies.

Authors' Disclosures

No disclosures were reported.

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