Editorial

Stem Cell INVESTIGATION

Human acute leukemia induced pluripotent stem cells: a unique model for investigating disease development and pathogenesis

Clara Bueno[1,2], Pablo Menendez[1,2,3]

[1]Josep Carreras Leukemia Research Institute and Department of Biomedicine, School of Medicine, University of Barcelona, Barcelona, Spain; [2]Centro de Investigación Biomédica en Red de Cáncer (CIBER-ONC), ISCIII, Barcelona, Spain; [3]Instituciò Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain

Correspondence to: Pablo Menendez, PhD. Josep Carreras Leukemia Research Institute, School of Medicine, University of Barcelona, Casanova 143, 08036, Barcelona, Spain. Email: pmenendez@carrerasresearch.org.

Provenance: This is an invited Editorial commissioned by Editor-in-Chief Zhizhuang Joe Zhao (Pathology Graduate Program, University of Oklahoma Health Sciences Center, Oklahoma City, USA).

Comment on: Chao MP, Gentles AJ, Chatterjee S, *et al*. Human AML-iPSCs reacquire leukemic properties after differentiation and model clonal variation of disease. Cell Stem Cell 2017;20:329-344.e7.

Received: 19 May 2017; Accepted: 26 May 2017; Published: 13 June 2017.

doi: 10.21037/sci.2017.05.12

View this article at: http://dx.doi.org/10.21037/sci.2017.05.12

Human cancer is generally studied once the full transformation events have taken place. Accordingly, investigations of key questions about early cancer pathogenesis, such as cell-of-origin, target cell for transformation, developmental impact of cancer-specific genetic insults, lineage affiliation of fusion genes and intratumor heterogeneity, are not amenable using patient samples (1,2). It is becoming increasingly clear that for almost all cancers, the cancer bulk displays features of normal tissue organization, where cancer stem cells (CSCs) sit at the top of the hierarchical structure and drive tumor growth and evolution (3). Therefore, the establishment of effective disease models to study the developmental impact of cancer-specific genetic aberrations on human stem cell fate is in high demand.

Since its discovery in 2006 by Yamanaka and colleagues (4), reprogramming somatic cells to induced pluripotent stem cells (iPSCs) has shown unprecedented promise for the generation of custom-tailored cells for disease modeling, drug-screening, cell therapy and developmental biology (5,6). iPSCs are a powerful tool for modeling key aspects of human disease that cannot otherwise be addressed by patient sample analyses or animal models (5,6). Because leukemia manifests as a developmental cell blockage, the generation and differentiation of leukemia-specific iPSCs offers a promising *in vitro* model to study the earliest events leading to the specification of both normal and malignant hematopoietic tissue, and to unravel the molecular and cellular mechanisms underlying the pathogenesis of human leukemia. iPSCs are easily generated from healthy and diseased, patient-specific, cells at different developmental stages (7). However, reprogramming human primary cancer cells remains challenging, and only a few reports have demonstrated successful reprogramming of malignant cells. Indeed, iPSCs from primary leukemic cells have been generated only from chronic hematological malignances, including Philadelphia+ CML, PMF, JAK2-V617F+ PV and JMML (8-10). The generation of iPSCs from acute myeloid (AML) or acute lymphoid leukemias (ALL) has not been reported to date, whereas iPSCs have been generated from normal human myeloid, T-cells and B-cells (11). Our laboratory has recently demonstrated that despite multiple *in vitro* and *ex vivo* combinations of reprogramming factors using diverse delivery strategies together with transcriptomic and epigenetic "booster" reprogramming strategies, neither primary B-ALL blasts nor B-ALL cell lines could be reprogrammed to pluripotency (12). Functional assays coupled with global transcriptome and DNA methylome profiling suggested a developmental/differentiation refractoriness of human B-ALL to reprogramming to pluripotency. Thus, it remains an open question whether biological or technical reprogramming

barriers underlie the inability of B-ALL leukemic blasts to be reprogrammed.

This situation has now changed. In the March 2017 issue of *Cell Stem Cell*, Chao and colleagues published a seminal paper describing the generation of iPSCs from primary AML samples harboring MLL rearrangements (AML-iPSCs) (13). The generated AML-iPSCs retained AML-specific cytogenetic and molecular abnormalities but reset AML epigenetic marks. When used as a disease model to study the developmental impact of AML-specific (epi)-genetic aberrations, AML-iPSCs successfully recapitulated many aspects of the disease that were dependent on the differentiation context of the cell (13). Differentiation of AML-iPSCs into hematopoietic cells led to leukemic reacquisition, whereas non-hematopoietic lineages did not form tumors. Differentiating AML-iPSCs presented a leukemic-like myeloid-restricted phenotype with serial clonogenic potential *in vitro*. Moreover, AML-iPSC blood derivatives gave rise to an aggressive AML *in vivo*. Importantly, AML-associated transcriptome and DNA methylation signatures that were initially erased through reprogramming were reacquired upon blood re-differentiation, and this was not due to residual epigenetic memory present upon AML reprogramming to iPSCs. The authors also investigated the utility of AML-iPSCs as a model for characterizing and therapeutically targeting distinct AML subclones present in the tumor. This is clinically very relevant for the development of therapeutic interventions, as it is critical to target as many subclones as possible, and preferentially those cells harboring initiating oncogenic drivers, which are key substrates for therapeutic failure through the ability of specific clones, especially those carrying the oncogenic initiating event, to become refractory to therapy.

The study by Chao *et al*. (13) opens up new avenues in leukemia research and stem cell biology. Compelling evidence links reprogramming, pluripotency, lineage-specification and oncogenic transformation as closely intertwined processes (14). For example, leukemic mutations in oncogenes/tumor suppressors or chromosomal rearrangements leading to constitutive expression of chimeric proteins are commonly affiliated with a specific hematopoietic lineage and/or cell stage, indicating that the effects of leukemia-relevant mutations are highly influenced by environment and the differentiation state of a given cell. Of note, poorly differentiated aggressive human cancers express high levels of pluripotency-associated factors, suggesting that reprogramming to a more dedifferentiated state occurs during tumor progression. A fascinating challenge in cancer cell biology is to deconstruct the stepwise mechanisms by which cancer-specific mutations transform into pre-cancerous states followed by rapid transition to overt disease. In this setting, the availability of banked patient-specific cancer cells and cord blood units from newborns who later develop childhood cancer will also offer a unique opportunity to dissect the developmental and molecular mechanisms underlying the stepwise transformation process from a pre-cancerous to an overt cancer clone. A corollary to the reversal of the malignant phenotype to a pluripotent state is that if a cancer cell can be fully reprogrammed it would suggest that the so-called "cancer state" is not irreversible, a concept that would have significant implications for cancer biology (9). Furthermore, compound screening assays aimed at identifying targeted and effective treatments may be undertaken using differentiating derivatives of cancer cell-specific iPSCs. For instance, if the oncogenic mutations present in cancer cell-iPSCs prevent cellular differentiation, differentiation-inducing compounds that can cause tumor regression, such as all-trans retinoid acid in the case of acute promyelocytic leukemia, may be assayed.

A stumbling block for advancing iPSC-based leukemia disease models is the extremely low reprogramming frequency of primary leukemic cells and cell lines (7), which prevents prospective genotype-phenotype studies comparing a large cohort of cancer-iPSCs from adult and pediatric human cancers carrying different genetic composites. Consistent with the developmental refractoriness of human B-cell ALL to reprogramming to pluripotency (7), the work by Chao *et al.* reports the generation of AML-iPSCs from just two patients, further emphasizing the notion that reprogramming of primary leukemias is not a trivial task. Whether biological or technical reprogramming barriers underlie the inability of primary acute leukemic blasts to be reprogrammed remains to be explored. The differences in reprogramming MLL-rearranged B-ALL and AML suggest that epigenetic reprogramming may be dependent on leukemia tumor subtype and the specific genetic insults (13).

Active cell proliferation is key for transcription factor-induced cell fate change during cellular reprogramming, and acute leukemias barely/do not proliferate *in vitro*, making leukemia cell reprogramming an extremely difficult endeavor. To contend with this, our laboratory and others attempted to reprogram acute leukemic cell lines and primary blasts that were induced to proliferate through xenograft expansion, both before and after transduction with

Yamanaka factors. These approaches consistently failed to reprogram *in vivo*-expanded leukemic blasts, whereas EBV-immortalized healthy B-cells as well as healthy pro-B and pre-B precursors could be successfully reprogrammed (7), suggesting that the leukemia-initiating genetic event might represent a reprogramming barrier. The discovery/optimization of technical advances that allow at least short-term *in vitro* expansion of primary leukemic blasts would undoubtedly be a major step towards leukemia cell reprogramming (15). In fact, Chao and colleagues reported an instrumental contribution of human-stromal cells in facilitating *in vitro* expansion of their AML blasts that despite being quite limited and short-term, it eventually helped the proliferation-dependent early phases of the reprogramming process.

Recent studies on the three-dimensional chromosome regulatory landscape of human iPSCs have revealed the importance of chromosome structure and topologically associating domains in maintaining pluripotency (16). In addition, an elegant study has suggested that some large genetic rearrangements (e.g., ring chromosomes) are not compatible with the pluripotent state, likely due to the inability to establish the characteristic chromosomal topology of iPSCs (17). Whether genetically-complex leukemic chromosomal translocations impede leukemic blast reprogramming cannot be ruled out. Furthermore, these genetic rearrangements may simply be lethal in a pluripotent cell context. Preliminary data from our laboratory suggest that after generating a t(11;22) EWSR1-FLI1$_+$ translocation in human stem cells using CRISPR/Cas9 system (18), t(11;21)-carrying clones are lost after a few passages, reinforcing that the nature of the target cell of transformation is crucial and therefore cancer-specific mutations have to initially occur in the right cellular context to confer a phenotype (19,20). Further work is thus needed to identify and overcome the biological barriers impeding reprogramming of acute leukemias and primary cancer cells. Whether different genetic/cytogenetic subtypes of acute lymphoid and myeloid leukemias can be equally reprogrammed should be determined in future studies in order to provide a universal procedure to establish iPSCs from primary acute leukemias that recapitulate the developmental impact of leukemia-specific (epi)genetic insults for disease initiation, evolution and drug resistance.

Acknowledgements

Funding: P Menendez laboratory is supported by the European Research Council (CoG-2014-646903), the Spanish Ministry of Economy and Competitiveness (SAF2016-80481-R1, RTC-2016-4603-1), the Asociación Española Contra el Cancer, the ISCIII (PI14-01191), the Obra Social La Caixa-Fundaciò Josep Carreras, and the Generalitat de Catalunya (SGR330 and SLT002/16/00299).

Footnote

Conflicts of Interest: P Menendez is an investigator of the Spanish Cell Therapy cooperative network (TERCEL). C Bueno has no conflicts of interest to declare.

References

1. Bueno C, Montes R, Catalina P, et al. Insights into the cellular origin and etiology of the infant pro-B acute lymphoblastic leukemia with MLL-AF4 rearrangement. Leukemia 2011;25:400-10.
2. Sanjuan-Pla A, Bueno C, Prieto C. Revisiting the biology of infant t(4;11)/MLL-AF4+ B-cell acute lymphoblastic leukemia. Blood 2015;126:2676-85.
3. Thomas D, Majeti R. Biology and relevance of human acute myeloid leukemia stem cells. Blood 2017;129:1577-85.
4. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblasts cultures by defined factors. Cell 2006;126:663-76.
5. Menendez P, Bueno C, Wang L. Human embryonic stem cells: A journey beyond cell replacement therapies. Cytotherapy 2006;8:530-41.
6. Wu SM, Hochedlinger K. Harnessing the potential of induced pluripotent stem cells for regenerative medicine. Nat Cell Biol 2011;13:497-505.
7. Muñoz-López Á, van Roon EH, Romero-Moya D, et al. Cellular Ontogeny and Hierarchy Influence the Reprogramming Efficiency of Human B Cells into Induced Pluripotent Stem Cells. Stem Cells 2016;34:581-87.
8. Curry EL, Moad M, Robson CN, et al. Using induced pluripotent stem cells as a tool for modelling carcinogenesis. World J Stem Cells 2015;7:461-69.
9. Ramos-Mejia V, Fraga MF, Menendez P. iPSCs from cancer cells: challenges and opportunities. Trends Mol Med 2012;18: 245-47.
10. Yilmazer A, de Lazaro I. Taheri H. Reprogramming cancer cells: a novel approach for cancer therapy or a tool for disease-modeling?. Cancer Lett 2015;369:1-8.
11. Bueno C, Sardina JL, Di Stefano B, et al. Reprogramming human B cells into induced pluripotent stem cells and its

enhancement by C/EBPalpha. Leukemia 2016;30:674-82.
12. Muñoz-López A, Romero-Moya D, Prieto C, et al. Development Refractoriness of MLL-Rearranged Human B Cell Acute Leukemias to Reprogramming into Pluripotency. Stem Cell Reports 2016;7:602-18.
13. Chao MP, Gentles AJ, Chatterjee S, et al. Human AML-iPSCs reacquire leukemic properties after differentiation and model clonal variation of disease. Cell Stem Cell 2017;20:329-344.e7.
14. Banito A, Gil J. Induced pluripotent stem cells and senescence: learning the biology to improve the technology. EMBO Rep 2010;11:353-59.
15. Pal D, Blair HJ, Elder A, et al. Long-term in vitro maintenance of clonal abundance and leukaemia-initiating potential in acute lymphoblastic leukaemia. Leukemia 2016;30:1691-700.
16. Ji X, Dadon DB, Powell BE, et al. 3D Chromosome Regulatory Landscape of Human Pluripotent Cells. Cell Stem Cell 2016;18:262-75.
17. Bershteyn M, Hayashi Y, Desachy G, et al. Cell-autonomous correction of ring chromosomes in human induced pluripotent stem cells. Nature 2014;507:99-103.
18. Torres-Ruiz R, Martinez-Lage M, Martin MC, et al. Efficient Recreation of t(11;22) EWSR1-FLI1+ in Human Stem Cells Using CRISPR/Cas9. Stem Cell Reports 2017;8:1408-20.
19. Rodriguez R, Tornín J, Suárez C, et al. Expression of FUS-CHOP fusion protein in immortalized/transformed human mesenchymal stromal/stem cells drives mixoid liposarcoma formation. Stem Cells 2013;31:2061-72.
20. Rodriguez R, Rubio R, Gutierrez-Aranda I, et al. Expression of the FUS-CHOP fusion protein coupled to p53 deficiency induces liposarcoma in fat-derived murine but not human Mesenchymal Stem Cells. Stem Cells 2011;29:179-92.

doi: 10.21037/sci.2017.05.12
Cite this article as: Bueno C, Menendez P. Human acute leukemia induced pluripotent stem cells: a unique model for investigating disease development and pathogenesis. Stem Cell Investig 2017;4:55.

