The Pediatric Acute Leukemia Fusion Oncogene ETO2-GLIS2 Increases Self-Renewal and Alters Differentiation in a Human Induced Pluripotent Stem Cells-Derived Model

Salvatore Nicola Bertuccio1,2, Fabien Boudia2,3, Marie Cambot2,4, Cécile K. Lopez2,3, Larissa Lordier5, Alessandro Donada6, Eille Robert7, Cécile Thrant7, Zakia Aid2, Salvatore Serravalle1, Annalisa Astolfi8, Valentina Indio8, Franco Locatelli1, Andrea Pession1, William Vainchenker2,4,5,8, Riccardo Masetti1, Hana Raslova2,8,9, Thomas Mercher2,3,8,9

Correspondence: Thomas Mercher (e-mail: thomas.mercher@inserm.fr).

The ETO2-GLIS2 fusion is associated with young and aggressive pediatric acute megakaryoblastic leukemia (AMKL) and is thought to arise in utero. To investigate ETO2-GLIS2 consequences in human hematopoietic cells and overcome the difficulty to access appropriate fetal stages, we engineered induced pluripotent stem cells (iPSC) to obtain hematopoietic-specific ETO2-GLIS2 expression and analyzed in vitro hematopoietic differentiation of iPSC toward the megakaryocytic lineage, which presents common features with human hematopoiesis during early development. As compared to controls, ETO2-GLIS2 expression induced an increased proportion of CD41+42+ megakaryocytes at several differentiation timepoints and generated a CD41low42low population that is absent in controls. In addition, ETO2-GLIS2 enhanced proliferation and self-renewal capacities in methylcellulose assays. To understand the molecular consequences of ETO2-GLIS2 expression in human progenitors, we next performed RNA sequencing on flow purified CD41+42+ megakaryocytes and the abnormal CD41low42low population. Compared to wild-type CD41+42+ profiles, ETO2-GLIS2-expressing CD41+42+ profiles were enriched for several ETO2-GLIS2-dependent transcription factors, were not deregulated to the same extent as in patients’ blasts. Together, this human ETO2-GLIS2 iPSC model recapitulates differentiation alterations, increased self-renewal and transcriptional signatures observed in human AMKL and should therefore represent an interesting platform to perform future molecular and preclinical investigations.
with a low number of additional genetic alterations and a dismal prognosis. It induces a deregulation in the balance between hematopoietic master regulators, including a higher expression of ERG and GATA3 associated with a drastic reduction in GATA1 activity in human AMKL cells. However, the consequences of ETO2-GLIS2 expression in normal human hematopoietic cells and its leukemogenic potential is unknown to date. In several instances, de novo AMKL patients are diagnosed few weeks/months after birth and in twins, suggesting that the fusions were generated in a hematopoietic stem or progenitor cell during early fetal development. Based on the observation that normal megakaryocyte differentiation from human embryonic stem cells resemble early ontogenic stages, we reasoned that hematopoietic differentiation derived from human induced pluripotent stem cells (iPSC) represents a developmentally-relevant context to model pediatric-specific AMKL fusion oncogenes.

To this aim, we used normal iPSC derived from healthy hematopoietic progenitors to perform a targeted knock-in at the AAVS1 locus, through zinc-finger nucleases-mediated recombination, to introduce a GFP-tagged ETO2-GLIS2 fusion gene under the transcriptional control of the pan-hematopoietic human CD43 promoter (Fig. 1A). Importantly, this allows to assess ETO2-GLIS2 function independently of iPSC line-to-line variances. We identified 23 clones with homozygous integration (Supplemental Fig. 1A and supplemental Table 1, Supplemental Digital Content 1 and 2, http://links.lww.com/HS/A54) and were thereafter compared to the parental control iPSC line. Functional expression of ETO2-GLIS2 upon hematopoietic differentiation of iPSC was validated through quantitative RT-PCR of ETO2-GLIS2 (Fig. 1B), through ETO2-GLIS2 protein detection (assessed by GFP in the nucleus) and through higher expression of the known fusion target gene GATA3 in hematopoietic progenitors at day 15 of differentiation, as compared to control iPSC-derived cells (Supplemental Fig. 1E,F, Supplemental Digital Content 1, http://links.lww.com/HS/A54).

Using an updated version of the hematopoietic differentiation protocol of iPSC described by Chou et al., (Supplemental Table 2, Supplemental Digital Content 2, http://links.lww.com/HS/A55), we assessed the consequences of ETO2-GLIS2 expression on megakaryocytic differentiation. From control iPSC clones, hematopoietic differentiation is observed by the detection of CD43+, CD34+, CD41+, and CD61+ following the acquisition of CD42 surface markers (Fig. 1C, 1D and Supplemental Fig. 2A, Supplemental Digital Content 1, http://links.lww.com/HS/A54). CD41+CD61+CD42+ represent progenitors while CD41+CD61+CD42− represent maturing megakaryocytes. From ETO2-GLIS2 iPSC clones, a higher fraction of megakaryocytic cells was reproducibly obtained with an increased percentage of CD41+CD42+ megakaryocytes as early as day13 and sustained at day 15 and day 18 of differentiation (Fig. 1C and E). Of note, ETO2-GLIS2 iPSC clones maintained a significant production of megakaryocytes over a longer period of time up to day 26, while the number of megakaryocytes generated from control iPSC clones declined after day 22 (data not shown). The ETO2-GLIS2 iPSC clones also showed an aberrant population characterized by low expression of CD41 and CD42 (named CD41lowCD42−) (Fig. 1C–E). Similarly to CD41, CD61 surface expression was significantly lower in both the ETO2-GLIS2-expressing CD41+CD42+ and, to a higher extent, the CD41lowCD42low populations, as compared to control cells (Fig. 1D and Supplemental Fig. 2B,C, Supplemental Digital Content 1, http://links.lww.com/HS/A54). These results indicated that ETO2-GLIS2 expression in human iPSC-derived hematopoietic cells induced differentiation alterations.

We then assessed the proliferation and self-renewal properties of ETO2-GLIS2-expressing progenitors. Firstly, we sorted 50,000 CD43+ hematopoietic cells from day 15 of differentiation into liquid culture. The output number of cells was counted after 4 days of culture and showed an 8-fold increase in the number of ETO2-GLIS2-expressing cells as compared to control cells (Fig. 1F and G). At that stage, morphological studies indicated clear proplatelets formation from controls megakaryocytes while ETO2-GLIS2-expressing megakaryocytes were larger and presenting with reduced signs of proplatelets formation (Fig. 1H). Secondly, day15 CD43+ hematopoietic cells were sorted into semi-solid medium to perform colony-forming assays scored after 7 to 10 days of culture and weekly serially-replated thereafter (Fig. 1I). While control cells formed colonies only in the first plates, ETO2-GLIS2-expressing cells formed larger colonies in the first plates (Fig. 1J) and could consistently form colonies up to the third replating, but not further (Fig. 1I). Importantly, flow cytometry analyses of ETO2-GLIS2-expressing cells after replating revealed an important proportion of GPA+ and CD43+ cells (Supplemental Figure 2D, Supplemental Digital Content 1, http://links.lww.com/HS/A54), supporting the idea of an erythroid differentiation. Of note, injection of day 15 or day 18 CD43+ cells from either control or ETO2-GLIS2 clones into irradiated immunodeficient NSG mice did not result in significant engraftment with a follow-up of over 9 months (data not shown) indicating that iPSC-derived progenitors obtained using this differentiation protocol are not maintained in vivo in these conditions. Together, these results showed that ETO2-GLIS2 expression enhances proliferation and self-renewal of iPSC-derived human hematopoietic progenitors in vitro but no long-term in vivo engraftment could be detected in conditioned recipients.

To characterize the molecular consequences of ETO2-GLIS2 expression in iPSC-derived cells, we performed a transcriptome analysis on control and the two ETO2-GLIS2 clones at day18 of differentiation. From the control, the CD41+CD42+ and CD41+CD42− cell populations were sorted to obtain normal immature progenitors and maturing megakaryocytes. From ETO2-GLIS2-expressing cells, CD41+CD42+ and the aberrant CD41lowCD42low were analyzed (Fig. 2A). As shown by tSNE and PCA plots, replicate conditions and cells from the two clones clustered together and apart from control cells (Fig.2B, Supplemental Fig. 3A, Supplemental Digital Content 1, http://links.lww.com/HS/A54). Gene set enrichment analyses revealed that ETO2-GLIS2 AMKL patient-derived gene expression signatures were enriched in ETO2-GLIS2-expressing cells (Fig. 2C, Supplemental Fig. 3B, Supplemental Digital Content 1, http://links.lww.com/HS/A54). Notably, ETO2-GLIS2-expressing CD41lowCD42low cells showed a significantly higher enrichment of these signatures as compared to ETO2-GLIS2-expressing CD41+CD42+ (Fig. 2C and D and Supplemental Table 3, Supplemental Digital Content 2, http://links.lww.com/HS/A55) and a significant loss of mature megakaryocyte features assessed by the xCell digital cellular deconvolution method or by gene ontology associated with a higher expression of ETO2-GLIS2 (Supplemental Fig. 3C,D,E,
Supplemental Digital Content 1, http://links.lww.com/HS/A54. These data indicate that ETO2-GLIS2-expressing CD41 low/C−D42 low cells present prominent transcriptional alterations that closely recapitulate AMKL patient leukemic cells signature. Expression of ETS and GATA factors are known to control proliferation of megakaryocytes obtained from iPSC16 and high ERG and low GATA1 activities are important for ETO2-GLIS2-transformed human blasts.9 Therefore, we quantified ERG and GATA1 expression in iPSC-derived hematopoietic cells at different timepoints (day 15, 18, and 22), in AMKL patients or AMKL patients-derived cell lines (eg MO7e) and in a previously described model of ETO2-GLIS2 ectopic expression in HEL cells.9 ERG presented a low basal expression without significant change in ETO2-GLIS2+ as compared to control cells (Supplemental Fig. 4A, Supplemental Digital Content 1, http://links.lww.com/HS/A54) and its expression was significantly lower in hematopoietic cells collected from day 22 ETO2-GLIS2+ iPSC-derived cells as compared to ETO2-GLIS2-expressing AMKL cells or HEL cells (Fig. 2E). Although GATA1 was moderately repressed by ETO2-GLIS2 at day 15, its expression surprisingly increased in ETO2-GLIS2+ cells at day 18 (Supplemental Fig. 4A, Supplemental Digital Content 1, http://links.lww.com/HS/A54) and was significantly higher in day 22 ETO2-GLIS2+ iPSC-derived cells compared to ETO2-GLIS2-
expressing AMKL cells or HEL cells (Fig. 2E). We then sorted CD41lowCD42low cells from day 22 ETO2-GLIS2+ and showed that they present a higher ERG and lower GATA1 expression compared to ETO2-GLIS2+ CD41+CD42+. However, none of these two populations reached the expression levels observed in the ETO2-GLIS2+ MO7e cell line (Supplemental Fig. 4B, Supplemental Digital Content 1, http://links.lww.com/HS/A54). These results demonstrated that ETO2-GLIS2-expressing human iPSC-derived progenitors obtained using this differentiation protocol globally recapitulate the transcriptional program observed in ETO2-GLIS2 AMKL patient leukemic blasts, but lack sustained deregulation of at least the 2 key transcription factors ERG and GATA1.

Here, we report the first model of ETO2-GLIS2 expression in human hematopoietic progenitors obtained from the precise gene editing of healthy iPSC. Our data demonstrate that ETO2-GLIS2 impairs normal megakaryocytic differentiation, increases self-renewal and globally recapitulates the transcriptional program observed in human AMKL leukemic blasts. Importantly, we identified in ETO2-GLIS2+ iPSC-derived hematopoietic cells relevant molecular differences associated with their differences in self-renewal and long-term growth properties as compared to human AMKL patient leukemic blasts. Indeed, ERG expression is not strongly induced in ETO2-GLIS2+ iPSC-derived progenitors and remains at a low level compared to its expression in ETO2-GLIS2 AMKL patient leukemic cells. In addition, the lack of sustained repression of GATA1 is consistent with the capacity of these cells to undergo erythroid differentiation upon serial replating and suggest that ETO2-GLIS2 does not fully block erythroid differentiation in these conditions. Therefore, we propose that the transient increase in self-renewal and altered differentiation in this human iPSC model of ETO2-GLIS2 expression results, in part, from an insufficient deregulation of the ERG/GATA1 activities. Of note, these characteristics could
be independent of ETO2-GLIS2 and may result from the type of hematopoiesis obtained with in vitro differentiation of iPSC to date. Indeed, it is generally accepted that current in vitro differentiation protocols do not reach an adult definitive hematopoiesis and it is still debated whether they recapitulate a primitive or transient definitive (EMP-like) hematopoiesis. Whether the strong expression of CD43 in the primitive streak may explain the results obtained here and would warrant the use of a different strategy to control ETO2-GLIS2 expression or whether a different differentiation protocol will be required to obtain definitive hematopoiesis and full-blown transformation should be interesting areas of investigation. Together, this modeling approach in iPSC represents an important tool to understand the cellular and molecular bases for the transition between normal to malignant hematopoiesis, to develop more faithful models of childhood leukemia using human cells and to perform preclinical drug testing for the development of novel therapeutic strategies.

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