LETTER TO THE EDITOR

Frequent and reliable engraftment of certain adult primary acute lymphoblastic leukemias in mice

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Acute lymphoblastic leukemia (ALL) represents a severe malignant disease of the hematopoietic system and novel treatment options are urgently needed, especially in adults, at relapse and in refractory disease [1,2]. Preclinical research on ALL is hampered by the fact that primary ALL cells from patients do not reliably grow in vitro, while established ALL cell lines often present additional alterations which are rare in patients [3]. In the individualized xenograft mouse model, primary ALL cells of different genetic backgrounds infinitively grow in severely immuno-compromised mice. The mouse model enables convenient work on surrogate patient-derived xenograft (PDX) ALL cells, including studies on leukemia stem cells and leukemia–stroma interaction [4]. While numerous pediatric PDX ALL models exist worldwide [5–7], engraftment of adult ALL cells appeared challenging so far [8]. Here, we show that primary adult ALL cells of two discrete high-risk cytogenetic groups reliably engraft in mice at high frequencies.

Primary leukemia cells were obtained from 15 adult ALL patients and transplanted into NSG mice (NOD/scid IL2 receptor gamma chain knockout mice; clinical data of patients in Table S1). All samples carried either the chromosomal translocation t(9;22) BCR/ABL (n = 10) or t(4;11) MLL/AF4 (n = 5) as both are associated with dismal prognosis in adult patients with ALL requiring better treatment options [9]. Samples were consecutively collected without selection except for the genetic translocation. Samples were mostly transplanted directly at the day of diagnosis without prior freezing/thawing; if unfeasible, cells were frozen and thawed under research conditions using optimized protocols [10]. Cells were transplanted into untreated animals in the absence of preconditioning by, e.g. chemotherapy or irradiation.

High and reliable engraftment was observed for primary adult ALL cells on NSG mice (Figures 1(A,B), S1(A,B)) and similarly in samples derived from peripheral blood or bone marrow aspiration (Table S2). 9/15 samples induced deadly leukemia in mice within 100 days (Figure 1(A)) which was associated with an infiltration of the bone marrow by human blasts of above 80%. Similar to pediatric ALL, adult ALL samples induced splenomegaly in mice, mostly of major extend enabling isolation of up to billions of PDX ALL cells per mouse (data not shown). Within 22 weeks and at the end of the observation period, 13/15 samples showed engraftment of at least 70% human cells in bone marrow (Figure 1(B)), 8 out of 10 t(9;22) and 5 out of 5 t(4;11) samples. 5/5 (100%) of the directly transplanted and 8/10 (80%) of frozen/thawed samples engrafted (Figure S1(A), Table S3), suggesting a potential loss of engraftment capacity due to freezing/thawing.

Our results are in contrast to published data describing low engraftment rates for primary adult t(9;22) BCR/ABL ALL samples unlike pediatric ALL samples, unless mice are pretreated by total body irradiation inducing unspecific inflammation in the bone

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marrow [8,11]. As we used similar ALL subtypes and identical mice as one study [8,11], underlying reasons remain elusive. In general, high quality sample retrieval represents a huge advantage for research accompanying clinical trials. We received primary samples within hours of diagnosis avoiding, e.g. overnight shipping required in certain multicenter trials. Pretreatment of mice by irradiation or chemotherapy might compensate for putative reduced cell viability, but other explanations are also conceivable.

In addition, frozen/thawed cells showed a tendency toward extended periods of time for cell passaging, defined as time from injection of ALL cells to deadly leukemia in mice (82.6 days in fresh versus 102.2 days frozen/thawed samples, in directly versus frozen/thawed samples). Over passaging, the time required for each passage slightly decreased in all samples, remaining constant thereafter. Two samples with slow growth in the first passage showed marked reduction in passaging time down to average levels within the first two passages (Figure 1(C)).

Taken together, engraftment rates of primary adult ALL samples in mice were very high in our hands and similar to those described for primary pediatric ALL samples [6,12].

PDX mouse models represent highly valuable tools for preclinical treatment trials and basic studies on disease biology [4,7]. Genetic engineering facilitates these studies as, e.g. bioluminescence in vivo imaging allows accurate follow up of disease development and treatment effects (Figures S2 and S3) [13,14]. For convenient wording, we propose referring to genetically engineered PDX models as ’GEPDX’ models in parallel to genetically engineered mouse models (GEMM).

Figure 1. Primary adult ALL samples engraft reliably in NSG mice and allow lentiviral transduction. (A,B) Time required for the first passage of individual PDX ALL samples is shown individually (A, numbers indicated unique patient numbers, UPN) or in cumulative form (B); samples after a freezing/thawing cycle are indicated with stripes or crosses. (C) Passaging time of individual samples with BCR-ABL rearrangement over several passages. Reduction of passaging time was statistically significant, comparing P0/P1 with P4/P5 in sample ALL-210 (from 132.8 to 42 days) and ALL-224 (from 114 to 79.3 days) according to two-tailed unpaired t-test. P, passage. (D) Cell viability after 5 days in culture of MLL-rearranged samples either freshly isolated from mice (plain) or after freezing/thawing (stripes). (E, F) PDX samples were lentivirally transduced, kept for 5 days in culture and measured for successful lentiviral transduction by flow cytometry on the recombinant fluorochrome. n.r.: non-rearranged.
While numerous GEPDX models exist for pediatric ALL, we aimed at establishing GEPDX models from adult ALL samples. In general, ALL cells are difficult to transduce and require lentiviral transduction which is facilitated by favorable cell viability in vitro. Unfortunately, some PDX ALL samples with MLL translocations exhibited limited viability of below 20% living cells after 5 days of culture which was inferior to PDX AML samples with MLL translocation and further aggravated upon freezing/thawing (Figures 1(D) and S4). Using our established protocols and constructs for expressing transgenes [13,14], adult PDX ALL cells with BCR/ABL or MLL rearrangement displayed very poor susceptibility toward lentiviruses with mean transduction efficiencies of around 5% (Figures 1(E) and S5). In adult PDX leukemia cells with MLL rearrangement, ALL were markedly less prone toward lentiviral transduction than AML cells (5 versus 50% transduction efficiencies, respectively). Pediatric PDX ALL cells with MLL rearrangement showed similar minor transduction rates as adult cells which is in sharp contrast to the favorable transduction efficiency of above 30% in pediatric PDX ALL samples without MLL rearrangement (Figure 1(F)). However, no correlation was detected between cell viability and transduction efficiency. PDX AML samples used in this study were representative established samples of the lab with representative transduction rates.

Nevertheless and despite these challenges, all adult PDX ALL cells allowed lentiviral transduction and enabled establishing GEPDX models, especially as enrichment of transgenic cells was performed by flow cytometry using a fluorochrome as molecular marker [13,14]. As lentivirally transduced samples allowed re-transplantation with similar growth kinetic, transgenic samples had retained stemness and in vivo growth behavior.

In summary, our data indicate that primary adult ALL samples engraft at similar rates in NSG mice as pediatric ALL samples. Although adult ALL cells with BCR-ABL or MLL-translocations reveal low survival in vitro and low lentiviral transduction rates, genetic engineering is feasible in PDX cells of all types of ALL enabling molecular studies. GEPDX models of acute leukemia might enable a better understanding of disease biology as well as precise preclinical treatment trials which will allow preclinical development of more effective therapies for the benefit of patients with acute leukemia in the future.

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Appendix: Materials and Methods

Handling of fresh primary ALL samples

Fresh primary samples arrived at our research facility typically as unmanipulated heparinized bone marrow suspension within 6 h after bone marrow aspiration. Leukemia cells were enriched by Ficoll gradient centrifugation and washed twice in PBS containing 1% fetal calf serum. Cells were frozen in 90% fetal calf serum with 10% DMSO in an isopropanol containing carrier allowing slow temperature decrease [10].

The presence of chromosomal rearrangements was confirmed by both FISH and PCR analysis in most primary samples.

Cell transplantation and analysis of mice to determine engraftment

From 15 adult ALL patient samples, 10^6–10^7 primary tumor cells per sample were transplanted into 2–4 NOD/scid gamma (NSG) mice of 6–12 weeks of age by tail vein injection. Engraftment was determined by staining of human CD45, CD38, CD19 (Biolegends, 302205) and CD7 (Life Technologies, MHC0705) in blood, bone marrow and spleen isolates as measured by flow cytometry [14]. Bone marrow and spleen typically contained >90% human cells at time of sacrifice. After successful engraftment of primary samples, patient-derived xenograft (PDX) cells were retransplanted into next generation recipients for up to four passages.

Flow cytometry analysis

PDX ALL and AML cells were genetically engineered using lentiviruses as described [13,14] to express fluorochromes. 10^6 cells were infected with concentrated lentivirus overnight in the presence of Polybrene (8 μg/ml; Sigma-Aldrich, St. Louis, MO, USA) in RPMI-1640 medium containing 20% fetal calf serum (FCS), 1% penicillin/streptomycin, 1% gentamycin and 2 mM glutamine. The following day, PDX cells were washed and cultivated 5 days in vitro in the same medium supplemented with 0.6% Insulin–Transferrin–Selenium (ITS), 1% sodium-pyruvate and 0.0434% α-Thioglycerol for ALL cells. Cell viability was analyzed by flow cytometry using forward and sideward scatter (FSC-SSC). Positive transduction rate was measured by flow cytometry.

Statistics

All statistical analyses were calculated with a two-tailed unpaired t-test using GraphPad Prism 6 software.