BITS :: Call for Abstracts 2023 - Oral communication

Type	Oral communication
Session	Algorithms for Bioinformatics
Title	Unraveling the Effect of Proliferative Stress in Vivo in Hematopoietic Stem Cell Gene Therapy
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Motivation

The hematopoietic system of patients enrolled in hematopoietic stem cells (HSC) gene therapy (GT) treatment is fully reconstituted upon autologous transplantation of engineered stem cells. Lentivirus (LV) is widely used in HSC-GT for the treatment of inherited disorders1. These vectors integrate semi-randomly into the genome of the targeted cells and the identification of vector integration site (IS) has been demonstrated to be an invaluable molecular marker for clonal identity, inherited by all HSC progeny, to uncover lineage dynamics in vivo at single-cell level. The number of active HSC clones repopulating the hematopoietic system ranges from 1-50.000, with the lower bound estimated not including HSC subclones generated from symmetric divisions. HSCs highly proliferate up to full restoration of homeostasis and compete for niche homing and engraftment. The impact of the proliferation stress in HSC on genetic instability2 remains an open question that cured patients advocate for characterizing long-term safety and efficacy. To this regard, the accumulation of somatic mutations has been widely used as a sensor of proliferative stress3. Somatic mutations can be used to address both open issues, as labels of subclones from the common HSC and as sensors of proliferative stress and aging.

Methods

The VISPA2 pipeline4 is used to identify and annotate IS from hematopoietic cells collected from patients and preclinical models that underwent HSC-GT application. The first input is a BAM file for each lineage and timepoint from which, using SAMtools, we remove optical and PCR duplicates that would lean to possible misassignment of variants or change in the Variant Allele Frequency (VAF). Then, each read is assigned to the correspondent IS to be able to call variants on each single clone, thanks to the fact that each LV integrates semi-randomly. SNP and indels are called using VarScan2 with optimized thresholds and parameters obtained from trial-and-error method and comparison with available Whole Genome Sequencing data. At this point, some important filters are applied with a de novo R script to remove low informative samples and germline mutations: removal of low abundant IS (coverage < 5 reads), samples with less than 10 IS retrieved, samples with less than 30 ng of DNA, mutations with a VAF over 95 considered to be germline (knowing that, due to our PCR approach, we are looking only to one allele). The accumulations of mutations over time and lineage are finally analyzed by a new index, the Mutation Index (MI), that normalizes the number of variations by the number of IS and the coverage.

Results

We characterized the proliferative stress of HSCs and their progeny over time by measuring the accumulation of mutations in the DNA of each IS. To test the feasibility of the approach, we took advantage of an experimental framework that combines tumor-prone Cdkn2a-/- and wild-type (WT) mouse models of HSC-GT. The Cdkn2a-/- mouse model provided the experimental conditions to detect the accumulation of somatic mutations, since the absence of p16INK4A and p19ARF enhances the proliferative potential of cells that have acquired oncogenic mutations. IS have been collected over time from different hematopoietic cell lineages after transplantation of Cdkn2a-/- or WT HSCs transduced with a genotoxic LV (LV.SF.LTR) or a GT-like non-genotoxic LV (SIN.LV.PGK)5. Indeed, mice receiving Cdkn2a-/- HSC transduced with LV.SF.LTR developed tumors and died significantly earlier (p-value < 0.0001, logrank test) compared to those receiving Cdkn2a-/- HSC transduced with the SIN.LV.PGK.
Our results showed that mice receiving HSCs transduced with LV.SF.LTR have a higher MI compared to those receiving SIN.LV.PGK-transduced cells, reflecting the level of genotoxicity of the different vector used. Moreover, MI is significantly higher (p-value < 0.001, Anova test) and increased over time in mice receiving Cdkn2a-/- HSC compared to those that received WT cells, reflecting the proven higher DNA fragility of Cdkn2a-/- background when compared to the WT one. In agreement with the literature, somatic mutations were particularly enriched in myeloid cells as compared to the lymphoid ones.
Our results showed that for the first time, by combining the analysis of acquired mutations with IS, we can unravel HSC proliferation stress in vivo. We are now applying our model to different HSC-GT clinical trials, to potentially reveal HSCs proliferation stress in different inherited disorders, and to identify HSC sub-clonal trees based on their symmetric and asymmetric divisions. These studies will open the doors to in vivo non-invasive studies of HSC activity in humans.

Info

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Availability	-

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