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Type	Oral communication
Session	Algorithms for Bioinformatics
Title	Longitudinal analysis of the accumulation of somatic mutations in clonal tracking study in vivo revealed patterns of proliferative injuries in genotoxic mice
All Authors	Gazzo F(1,2), Cesana D(1), Gallina P(1), Pavesi G(2), Chiara M(2), Montini E(1), Calabria A(1)

Affiliation

(1) San Raffaele Telethon Institute for Gene Therapy, Ospedale San Raffaele, Milano
(2) Dipartimento di Bioscienze, Università degli Studi di Milano, Milano

Motivation

Hematopoietic stem cell (HSC) Gene therapy (GT) applications based on retroviral vectors are now effective treatments for several inherited monogenic diseases. Integration site (IS) studies are an invaluable tool to monitor the corrected HSC in vivo. However, IS data cannot reveal any information on the proliferative properties of the HSC over time, important parameters to evaluate the safety of GT. The hematopoietic system reconstitution after transplant induces HSC in proliferation stress, a potent driver of functional decline. Since the accumulation of genomic somatic mutations can be a sensor of the proliferative cell stress, we developed a new method to characterize at single cell granularity the proliferation stress of HSC and their progeny in the different phases of the hematopoietic reconstitution by the accumulation of mutations in the vector/host genome junction sequences. Once validated the approach in mice models, we will apply the method in clinical trial data to understand the dynamics of HSC and their differentiation capacity over time, improving the safety and efficacy of HSC GT.

Methods

To proof the feasibility of the hypothesis, we designed an experimental condition using a mouse study as preclinical G model. Different mutation rates can be identified in the different genetic backgrounds (Cdkn2a-/- vs WT) and genotoxic potential of the tested vector (genotoxic LV or GT-like SIN LVs) unravelling the accumulation rate in normal or stressed conditions, such as those occurring during malignant transformation. We used standard protocols to retrieve and identify IS and thus identify each HSPC clone and its progeny. After sonication based PCR procedures to extract vector-genome portions, we sequenced PCR fragments and identify IS with VISPA2 software. From each BAM file we called variants by applying both Varscan and Freebayes, thus obtaining the list of somatic variations in the genomic portion after each IS that are directly associated to that HSPC clone observed in all samples, lineages (Myeloid, Lymphoid T and B), and time point. We constructed a phenetic matrix with SNPs and Indels to visualize the evolution and accumulation of the mutations, intersecting data coming from both the software to understand which are the true mutations. We then removed false positive mutations by analysing (1) the genomic distance from the IS, (2) the sequence content (for example excluding homopolymers), and (3) by integrating known mutations from public databases.

Results

We designed and developed a novel framework to analyse the accumulation of somatic mutations over time and lineages in GT applications. We tested our method in a mouse study and preliminary results confirmed that the accumulation of mutations significantly occurred in tumour prone mice treated with genotoxic vector than in safe condition (PGK), thus proving the induction of DNA fragility. Moreover, we confirmed that one of the top mutated gene is Sfi1, a well-known and characterized gene in GT mice to study vector genotoxicity. We had also evidence that the major confirmed somatic mutations have an estimated variant allele frequency (VAF) near 50%, but we also found, as it has been demonstrated in literature, mutations with a VAF in the range of 10-35% linked with clonal expansions leading to tumours and thus could not have been inherited.
We are completing the method validation and testing to then apply the same procedure in clinical datasets to dissect the impact of proliferative stress in G treatment.

Info

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Figure

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Availability	-

Corresponding Author	
Name, Surname	Francesco, Gazzo
Email	gazzo.francesco@hsr.it
Submitted on	30.04.2021

Società Italiana di Bioinformatica
C.F. / P.IVA 97319460586
E-mail bits@bioinformatics.it

Sede legale Viale G. Mazzini, 114/B - 00195 Roma
Website bioinformatics.it