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Previews

Myeloproliferative Neoplasms: The Long Wait for *JAK2*-Mutant Clone Expansion

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Myeloproliferative neoplasms (MPNs) are hematological malignancies caused by somatic mutations originating from a single hematopoietic stem cell (HSC). In this issue of *Cell Stem Cell,* Van Egeren et al. (2021) used whole-genome sequencing of hematopoietic colonies to reconstruct the clonal history and time of acquisition of the disease-initiating gene mutation.

Myeloproliferative neoplasms (MPNs) are clonal hematological malignancies characterized by an overproduction of mature blood cells. Almost 90% of MPN patients harbor a somatic driver mutation in one of three genes: *JAK2*, *CALR*, or *MPL* (Vainchenker and Kralovics, 2017). *JAK2*-V617F is the most frequent driver gene mutation in MPN patients and is detectable in hematopoietic stem cells (HSCs). Transplantation of a single HSC that carries the *JAK2*-V617F mutation was sufficient to cause MPN in mouse models (Lundberg et al., 2014). This led to the concept that MPN is a clonal hematopoietic stem cell disease.

In the current issue of *Cell Stem Cell,* Van Egeren et al. (2021) reconstructed the clonal architecture and phylogeny of hematopoiesis in two patients with *JAK2*-V617F-mutated MPN. Since DNA sequencing of single HSCs cannot be done with high coverage, DNA from single HSC-derived hematopoietic colonies was used for whole-genome sequencing (WGS) (Figure 1). The unique patterns of somatic sequence alterations for each HSC-derived colony were used to reconstruct the divisional history and relatedness of the HSCs. The somatic mutation rate was calculated by dividing the average number of somatic mutations per cell by the age of the patient and a value of 19 mutations per year was obtained for both patients. This allowed a timeline for the branching of the HSC mutational history to be established. The methodology was previously successfully applied to reconstruct the hematopoiesis dynamics in a healthy 59-year-old man (Lee-Six et al., 2018).

Van Egeren et al. (2021) found that *JAK2* mutant and wild-type HSCs represent distinct clades. The age at which the *JAK2*-V617F mutation was acquired can be estimated from the number of sequence alterations common to all *JAK2* mutant colonies and the mutation rate. Importantly, this estimate is based on the average mutation rate of wild-type HSCs before they acquired *JAK2*-V617F and is not influenced by possible changes of the mutation rate in *JAK2* mutant HSCs. This led to the conclusion that the *JAK2*-V617F mutation was acquired 25 years and 40 years prior to sampling, which would correspond to age 9 and 23 years of their two MPN patients, respectively. Since extensive branching occurred within the *JAK2* mutant HSCs, the mutation rate can also be calculated for the *JAK2* mutant HSCs. Perhaps surprisingly, the estimated mutation rate in *JAK2* mutant HSCs was the same as in wild-type HSCs.

A similar study in MPN patients using the same approach was deposited on the bioRxiv server (Williams et al., 2020). WGS of DNA from single cell-derived colonies from 10 MPN patients with *JAK2* mutations was performed and the acquisition of the *JAK2*-V617F mutation was again estimated to have occurred several decades before MPN diagnosis, in some cases in childhood or even *in utero*. In contrast to Van Egeren et al. (2021), they found a higher mutation rate in *JAK2*-mutated HSC-derived colonies compared to *JAK2* wild-type colonies in six of their ten patients. Williams et al. (2020) also found shortened telomere length in *JAK2*-mutated colonies compared to wild-type, suggesting an increase cell division rate in *JAK2* mutant HSCs. However, eight of the ten MPN patients carried functionally relevant additional somatic mutations within the *JAK2* mutant colonies, whereas the two MPN patients studied by Van Egeren et al. (2021) carried no such additional mutations. In fact, the two patients in the study by Williams et al. (2020) who do not have additional functionally relevant somatic mutations also showed no difference in the mutation rate between wild-type and *JAK2* mutant HSCs. Together, this suggests that *JAK2*-V617F alone does not increase the mutation rate in HSCs. Given that *JAK2*-V617F HSCs must have divided more often than wild-type HSCs in order to expand the *JAK2* mutant clone, this poses an interesting and important question: how this is possible? Two possible explanations are that *JAK2* mutant HSCs are more prone to symmetrical HSC divisions than wild-type HSCs, or their mutation rate per cell division would have to be lower than wild-type HSCs.

Van Egeren et al. (2021) also reconstructed the population size history of the *JAK2*-V617F clone through a phylogenetic inference algorithm and the assumption that wild-type and *JAK2* mutant HSCs divide once per year (Lee-Six et al., 2018). The differences in growth rates of wild-type and *JAK2* mutant HSCs were inferred from a Wright-Fisher population genetics

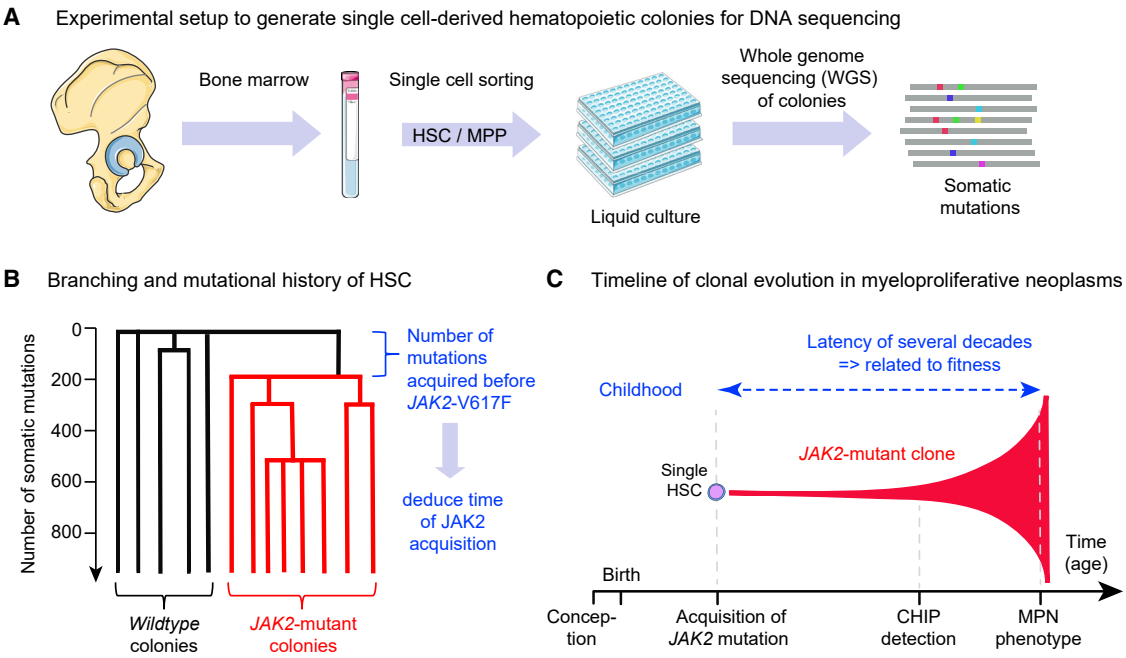

Figure 1. Reconstruction of the Clonal History in Myeloproliferative Neoplasms (MPNs)

(A) Schematic drawing of the experimental setup. Hematopoietic stem cells (HSCs) or early progenitors (MPP) were isolated from human bone marrow by FACS sorting, deposited as single cells in individual wells of microtiter plates and cultured *in vitro* to derive colonies of offspring cells. DNA extracted from single colonies was subjected to whole-genome sequencing.

(B) The divisional history and relatedness of the HSCs can be reconstructed from the unique patterns of somatic sequence variations between colonies. The somatic mutation rate was calculated as the average number of somatic mutations per HSC divided by the age of the patient. The age when the *JAK2* mutation was acquired can be estimated from the number of somatic mutations shared by all *JAK2* mutant colonies divided by the mutational rate.

(C) Both studies (Van Egeren et al., 2021; Williams et al., 2020) concluded that the *JAK2*-V617F driver mutation was acquired decades before the diagnosis of MPN. The *JAK2* mutant clone can be detected in peripheral blood as "clonal hematopoiesis of indeterminate potential" (CHIP) and only a small fraction of individuals with CHIP progress to MPN disease.

model first written in 1931, which captures genetic drift as well as the action of natural selection. Here the authors compared the genealogies produced by simulating this model to those observed under phylogenetic reconstruction of the HSC divisions and found an inferred relative fitness of *JAK2*-mutated HSCs over wild-type HSCs of 63% ± 15% and 44% ± 13% in the two patients studied. Using an independent modeling approach, Williams et al. (2020) found relative fitness to range from 43% to 68% in the two MPN patients with no additional functionally relevant somatic mutations and higher values in patients with additional somatic mutations. This heterogeneity of fitness may influence disease latency, and the factors underlying this heterogeneity need to be defined to better understand how and when overt disease arises.

In 2014, several reports described the frequent occurrence of somatic mutations in peripheral blood cells of healthy individuals, referred to as "clonal hematopoiesis of indeterminate potential" (CHIP) (Jaiswal and Ebert, 2019). Of note, the *JAK2*-V617F mutation was one of the most frequent mutations found in CHIP studies and prevalence of *JAK2*-V617F reached 3.5% in a general population using ultra-sensitive PCR methods (Cordua et al., 2019). Nevertheless, the relative fitness in healthy individuals with *JAK2*-mutated CHIP was estimated to be 14.6% (Watson et al., 2020), which is lower than in the two studies with *JAK2*-mutated MPN patients. Thus, additional factors that increase the fitness of the *JAK2*-mutated clone might be necessary for the transition from CHIP to phenotypic MPN.

The studies by Van Egeren et al. (2021) and Williams et al. (2020) showing that the latency between acquisition of the driver gene mutation and manifestation of MPN is much longer than was generally assumed provide fascinating insights into the early steps in the pathogenesis of MPN. New questions arise and we need to learn more about the factors that influence the fate of the *JAK2* mutant clone during this very long latency.

DECLARATION OF INTERESTS

R.C.S. has consulted for and received honoraria from BMS/Celgene and Novartis. D.L.P. and P.A. declare no competing interests.

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Stabilizing Formative Pluripotent States with Germ Cell Competency

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Previous work demonstrating the existence of intermediate pluripotency states in post-implantation embryos had ignited a debate on whether "formative" pluripotency can be stabilized in pluripotent stem cell (PSC) lines. In this issue of *Cell Stem Cell*, two papers show that polarized epithelial and germ cell-competent formative PSCs can be maintained in modified activin-dependent conditions.

By definition, pluripotent stem cells (PSCs) should be able to form the three embryonic germ layers, plus the gametes or germ cells (Robinton and Daley, 2012). Although the first PSCs were derived from germ cell tumors, called teratocarcinomas, which form the three somatic germ layers avidly, there has been limited success in inducing primordial germ cell (PGC) fate directly from PSCs (Hayashi et al., 2011). Hence, assays for pluripotency always include verification of trilineage competency *in vivo* and *in vitro*, but only seldom verify competency for the germline—due to technical difficulties *in vivo* and technological obstacles *in vitro*.

The transient window of pluripotency *in vivo* stretches from blastocysts to gastrulation embryos in mammals. Dynamic changes in the mouse pluripotent epiblast occur during E4–E8, which also overlap with the time window during which embryos implant into the uterus. For historical reasons, PSCs established from ~E4 pre-implantation blastocysts are known as embryonic stem cells (ESCs), whereas PSCs generated from post-implantation epiblasts came to be called epiblast stem cells (EpiSCs) (Robinton and Daley, 2012). Interestingly, EpiSCs were later found to display transcriptomes more similar to the ~E7.5 anterior primitive streak during gastrulation, not the epiblast. The concepts of "naive" and "primed" pluripotency were introduced to describe and understand the myriad differences between the pre- and post-implantation stages of pluripotency (Smith, 2017). A variety of PSCs have been re-classified according to this conceptual scheme instead of their cell source. For example, EG cells derived from mouse PGCs are classified as naive PSCs, while human ESCs or iPSCs are classified as primed PSCs.

Transcriptomic and functional genomic studies dissecting the exit from naive pluripotency increasingly suggested that the transition from naive to primed pluripotency proceeds through an intermediate state of pluripotency (Cornacchia et al., 2019; Hayashi et al., 2011; Kalkan and Smith, 2014). Amidst a confusing array of intermediates, Smith and colleagues hypothesized the concept of "formative" pluripotency, which invoked similarity to the ~E5–E6 pre-streak epiblast, just before gastrulation (Smith, 2017). As cells exit naive pluripotency and acquire formative pluripotency, PSCs should (1) switch from an apolar state to a polarized epithelial state, (2) shut off naive transcription factors such as Nanog, Esrrb, and Klf4 and utilize formative factors instead (Kalkan et al., 2019; Smith, 2017), and (3) become directly responsive to cues to form PGCs and three germ layers, just like the pre-streak epiblast before gastrulation. Although Saitou and colleagues induced naive ESCs into epiblast-like cells (EpiLCs) via transient FGF/activin treatment (Hayashi et al., 2011) such that they resembled E5.75 epiblasts and became responsive to PGC induction, EpiLCs could not maintain self-renewal as stem cells. It was not possible to stabilize stem cell lines with formative pluripotency hitherto (Smith, 2017). Unlike primed PSCs, which are somewhat lineage restricted and heterogeneous, formative PSCs are expected to possess