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The Harmful Consequences of Increased Fitness in Hematopoietic Stem Cells

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Clonal hematopoiesis of indeterminate potential (CHIP) describes clonal selection of a hematopoietic stem cell with a somatic mutation that confers increased fitness, influenced by a selective environment such as aging, inflammation, or therapeutic exposure. In this issue of *Cell Stem Cell*, Hsu et al. (2018) explore the role of cytotoxic therapy in disease-relevant CHIP.

Clonal hematopoiesis of indeterminate potential, or CHIP, has received considerable attention in the past few years. Recent reports established direct links between pre-existing somatic mutations in hematopoietic stem cells (HSCs) and increased risks of developing myeloid malignancies, including therapy-related myeloid neoplasms (t-MNs). For example, *TP53* mutations are detectable in the blood of some t-MN patients before exposure to chemotherapy for the primary malignant disease, with the same mutation detected in the t-MN cells. These observations have led to a model in which mutated HSCs are conferred with a "fitness" advantage and preferentially survive and repopulate the hematopoietic compartment following chemotherapy and/or radiotherapy. Two new studies elegantly illustrate this model and demonstrate that this process may ultimately be preventable (Hsu et al, 2018; Kahn et al., 2018). In this issue of *Cell Stem Cell*, Hsu et al. address the role of *PPM1D* mutations in clonal hematopoiesis after two different cellular stressors: cytotoxic therapy and hematopoietic transplantation. Their findings emphasize the importance of elucidating the role of specific pre-existing mutations and treatment-mutation interactions in mediating increased risk for the development of t-MNs. This work may ultimately inform the choice of treatment for the primary malignant disease.

It is estimated that the development of CHIP may be inevitable with age (Zink et al., 2017) and with cytotoxic drug exposure (Wong et al., 2018). A few genes (*DNMT3A*, *TET2*, and *ASXL1*) comprise the majority of known mutated CHIP genes. However, mutations of hundreds of genes have been found in CHIP, most of which are not known drivers of myeloid malignancies. This presents a conundrum for researchers and clinicians: (1) which genes and mutations are pathogenic; (2) which are predictive of subsequent disease; and (3) what is the mechanism by which they grant HSCs a competitive advantage over non-mutated counterparts?

New findings reveal a class of CHIP genes with unique characteristics. Unlike forty other genes tested by Wong et al. (2018), DNA damage response (DDR) genes (*TP53*, *PPM1D*, *ATM*, *BRCC3*, *SRCAP*, and *RAD21*) are more prevalent in CHIP among individuals treated with cytotoxic therapy. In contrast, these genes were not selected for during regenerative hematopoiesis following autologous transplantation; *PPM1D* clones actually decreased in frequency (Wong et al., 2018). In comparison, *DNMT3A* and *TET2* mutations enable HSCs to outcompete wild-type counterparts in the absence of cytotoxic drugs, indicating that CHIP gene mutations have environment-specific phenotypes. An additional enigma is that, with the exception of *TP53*, mutations in DDR genes are less prevalent in t-MNs, compared to their prevalence in CHIP post-cytotoxic therapy (Wong et al., 2018). This counterintuitive finding suggests that while HSCs with DDR gene mutations are chemoresistant, most have low leukemogenic potential.

Among the DDR CHIP genes, *PPM1D* is of particular interest because it is recurrently mutated in t-MNs, yet the role of *PPM1D* mutations in t-MN pathogenesis is unclear. PPM1D (WIP1) is a protein phosphatase transcriptionally activated by TP53 in the DDR. PPM1D dephosphorylates DDR components, including ATM and TP53 itself, shutting down the DDR after DNA repair. *PPM1D* amplification or gain-of-function mutations occur in a number of solid tumors. Mutations in exon 6 prematurely truncate the C terminus and increase PPM1D half-life due to loss of proteasomal targeting and degradation (Kleiblova et al., 2013). Consequent PPM1D overexpression further suppresses TP53 and the DDR, overriding the cell cycle checkpoint and blocking apoptosis.

Kahn et al. (2018) report that while 0.5%–5% of unselected individuals harbor a *PPM1D* CHIP mutation, the frequency increases to 2%–18% in patients treated with chemotherapy for lymphoma. Hsu et al. identified *PPM1D* mutations in 0.3% of *de novo* myeloid neoplasms compared to 20% of t-MNs. These findings buttress the association of *PPM1D* mutations with cytotoxic exposures. Both studies confirm that *PPM1D* mutations cluster within exon 6, are truncating mutations, and increase the mutant protein level. Using sophisticated CRISPR-Cas9 gene editing technologies in human acute myeloid leukemia cell lines *in vitro* and *Ppm1d*-mutant primary mouse HSCs *in vivo*, the two groups showed that truncating *PPM1D/Ppm1d* mutations increased cell viability and competitiveness upon their exposure to commonly used chemotherapeutic agents. In contrast, *PPM1D/Ppm1d*

mutations had a negligible effect in the absence of cytotoxic chemotherapy and negative effect during the challenge of regenerative hematopoiesis.

Both studies report that *PPM1D* mutations attenuate the apoptotic response to chemotherapy. Kahn et al. also report a defect in cell-cycle arrest, not observed by Hsu et al. This discrepancy may be due to different chemotherapeutics used. Regardless, the cellular consequence of *PPM1D* mutation is distinct from that of other CHIP genes, which increase HSC self-renewal and/or proliferation. Clearly, our understanding of the impact of CHIP gene mutations remains incomplete. Which mutations confer a fitness advantage in the spectrum of selective pressures from normal processes (aging, infection, drugs, toxins, etc.) that individuals experience in the course of a lifetime? What is the mechanism in each context? This information is essential for the development of tailored approaches to prevent and treat myeloid malignancies.

Hsu et al. took steps to assess the leukemogenicity of *PPM1D* mutations. Of note, the median variant allele frequency (VAF) of *PPM1D* mutations in t-MNs is only 5%, consistent with the mutation being present in only a subclone of the tumor or an unrelated clone. In some patients, the *PPM1D* VAFs are much higher, likely present in most leukemia cells. This is evidenced by the identification of a *PPM1D* mutation in a xenograft established from the malignant cells of a t-MN patient. Future studies determining if the *Ppm1d*-truncation mutant mice generated by Hsu et al. have increased susceptibility to spontaneous or therapy-induced myeloid neoplasms are imperative to firmly establish the oncogenic role of *PPM1D*.

Although, in the same pathway, *PPM1D* mutations do not phenocopy *TP53* mutations, and mutations of both genes co-occur at a relatively high frequency in t-MNs. For instance, *TP53* mutations are associated with a complex karyotype and poor prognosis, whereas *PPM1D* mutations are not. We do not yet know whether these mutant genes co-occur in the same population of cells, nor do we know the distinct and overlapping features of *PPM1D* and *TP53* mutations in clonal expansion and leukemogenesis.

An enticing finding of both labs is that chemotherapy-induced selection of *PPM1D* mutant cells can be inhibited by a specific PPM1D inhibitor, GSK2830371. Although GSK2830371 has a short *in vivo* half-life, it has shown anti-tumor efficacy *in vivo* (Chen et al., 2016). One can imagine that temporary PPM1D inhibition may be chemopreventive in patients with CHIP, or it could prevent outgrowth of *PPM1D* mutant HSC clones during cytotoxic therapy for a primary malignancy, reducing the risk of t-MNs. Alternatively, PPM1D inhibitors may be useful for the treatment of myeloid neoplasms with *PPM1D* mutations.

Intriguingly, both studies report that *PPM1D* mutations are preferentially selected by certain chemotherapeutics, such as cisplatin and etoposide. But, Hsu et al. found that this was not true for vincristine or fluorouracil, suggesting that elevated genotoxicity and enhanced triggering of the TP53 DDR by some drugs, doses, or both may explain this difference. Indeed, genotoxic chemotherapeutics are more closely associated with t-MN development than other drugs. Perhaps emerging cytostatic therapies in cancer treatment, such as kinase inhibitors, will decrease the incidences of t-MNs going forward.

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