FLT3 dancing on the stem cell

Whether or not FLT3 mutations are present and expressed within a leukemic hematopoietic stem cell has engendered some controversy. New evidence has now been presented on this issue that could change the way we manage the disease in the future.

Insight from Mark Levis

Defining and characterizing the hematopoietic stem cell (HSC) has been an endeavor that has occupied more than one generation of experimental hematologists. An ongoing challenge has been to reconcile a functional definition of the HSC, encompassing the properties of self-renewal and multi-lineage potential, with a phenotypic definition, i.e., which cell surface markers can be used to identify the true HSC, and there has been no shortage of controversy over this particular topic. To some on the periphery of the field, the arguments back and forth over how to precisely define the HSC may seem like arguing how many angels can dance on the head of a pin. Unlike that ancient philosophical conundrum, however, this issue of defining the HSC is deadly serious. Our ability to understand and develop better, more refined treatments for diseases such as acute myeloid leukemia (AML) will depend on our understanding the deepest roots of the problem.

AML is an aggressive hematologic malignancy that, despite being treatable with well-defined chemotherapy regimens and allogeneic transplant, is ultimately fatal in over half of all cases (Döhner et al., 2017). AML could be described as the original "cancer stem cell" disease, as it was using AML samples that John Dick and colleagues performed their pioneering work demonstrating the existence of leukemia stem cells (Bonnet and Dick, 1997). As the stem cell theory of AML (and cancer) evolved, studies using next-generation sequencing (NGS) established that AML developed following a series of founder and cooperating mutations in a relatively restricted set of genes (Welch et al., 2012). One of the most commonly mutated genes in AML is *FLT3*, which encodes a receptor tyrosine kinase (Levis and Small, 2003). The most common type of *FLT3* mutation is an internal tandem duplication (FLT3-ITD), consisting of an in-frame insertion of amino acid sequence into the juxtamembrane domain of the receptor, which results in constitutive kinase activity. NGS studies using primary cells from AML patients have clearly established that FLT3-ITD mutations occur relatively late in leukemogenesis (Jan et al., 2012; Welch et al., 2012). AML patients with FLT3-ITD mutations present with a highly proliferative variant of the disease, and although they can achieve remission with induction chemotherapy, they have a high relapse rate and poor overall survival. Their clinical outcome appears to be improved if they undergo allogeneic transplant while in remission (Levis, 2013). As a constitutively activated kinase, FLT3 represented an obvious target for drug development, and the first kinase inhibitor, midostaurin, has been approved for use in this subset of AML patients (Levis, 2017). Several additional selective, potent FLT3 tyrosine kinase inhibitors (TKIs) have entered pivotal trials and seem likely to eventually be incorporated into standard treatment for these patients (Leick and Levis, 2017).

Mouse studies have been central to understanding the biology of AML and in particular the role of FLT3 in the disease. Introduction of an FLT3-ITD mutation into hematopoietic progenitor cells results in a myeloproliferative disease, but not acute leukemia (Li et al., 2008). When another mutation is combined with the FLT3 mutation in these murine models, an acute leukemia usually results (Kelly et al., 2002; Rau et al., 2014; Poitras et al., 2016). This model of an FLT3-ITD–driven myeloid disorder has been extensively studied by several groups around the world and has been used to explore combinations of different AML-associated mutations with FLT3-ITD mutations. A central conundrum in this field has been the role of the FLT3-ITD mutation in the function of the HSC. In AML, FLT3-ITD mutations appear to be present in the leukemia stem cell–enriched populations (Levis et al., 2005), and they certainly act as driver of the disease. However, long-term HSCs have been classically defined as lacking FLT3 expression, and the presence of cell surface FLT3 was thought to mark the transition from HSC to MPP (multi-potent progenitor; Boyer et al., 2012). However, in 2012, Chu and colleagues noted that unfractionated or lineage-depleted bone marrow cells from FLT3-ITD knock-in mice had markedly reduced engraftment capacity and a relative depletion of c-kit$^+$ sca-1$^+$ Lin$^-$ CD150$^+$CD48$^-$ cells (a phenotype which more stringently defines an HSC-enriched population; Chu et al., 2012). They demonstrated that this depletion was associated with increased FLT3-driven cell cycling within this compartment and that inhibiting FLT3 signaling with a TKI reversed the effect. Their findings indicated that the mu-

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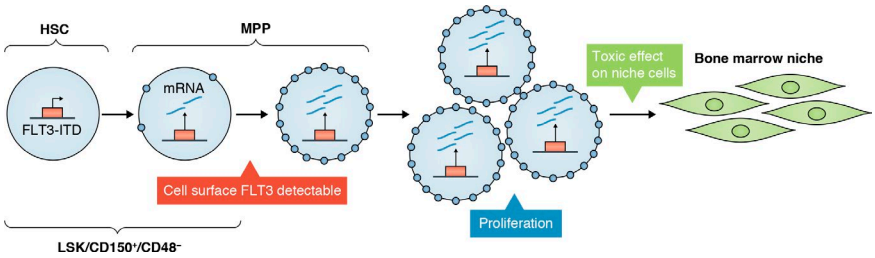
tated receptor was expressed in the HSC and influenced its function, and they suggested that HSCs may simply express cell surface FLT3 at a level below the limit of detection by flow cytometry. A crucial experiment performed in this study was one in which Lin^- bone marrow cells from both WT and FLT3-ITD knock-in marrow were mixed and competitively transplanted. Recipient marrow showed a decrease in Lin^- $CD150^+$ $CD48^-$ cells only in the FLT3-ITD fraction. WT Lin^- $CD150^+$ $CD48^-$ cells were present in normal numbers in the engrafted mice, consistent with a cell-intrinsic effect of the FLT3-ITD mutation, and again indicating that the mutated FLT3 was expressed and active in the HSCs.

In this issue of *The Journal of Experimental Medicine*, Mead et al. present new data that lead to a different interpretation of these findings. They performed single-cell mRNA analysis using FACS to isolate individual stringently defined HSCs and showed very low or absent FLT3 expression in these cells. Furthermore, mRNA expression analysis in these single cells revealed a more stem cell–like pattern in the cells lacking FLT3 expression. This suggests that within the stringently defined HSC population there exists "contaminating" MPPs because of a phenotypic overlap between HSCs and MPPs. The presence of FLT3 transcript in any LSK/$CD150^+$/$CD48^-$ cell marks one that has functionally transitioned from HSC to MPP, but retains the flow cytometric phenotype of an HSC. When they performed a competitive transplant experiment with a mixture of FLT3-ITD knock-in cells and FLT3 WT cells, they used fetal liver cells isolated from E15 embryos as a source of HSC-enriched cells. They found essentially the opposite result compared with Chu et al. (2012), namely that both FLT3-ITD and WT HSC-enriched populations were decreased. Why the opposite result? The authors argue that for a competitive transplant experiment to be valid, equal numbers of HSCs need to be used and that Chu et al. (2012) used an HSC-enriched population from FLT3-ITD mice that had likely had contaminating MPPs. By using fetal liver–derived phenotypic HSCs (which contain a normal fraction of LSK/$CD150^+$/$CD48^-$), there was a higher concentration of "true" HSCs from the FLT3-ITD mice and therefore a more valid competitive transplant.

If we accept these arguments, we can draw two conclusions from their findings. First, that the true HSCs from FLT3-ITD mice lack expression of the mutant receptor and, second, that the MPP population within these animals exert a cell-extrinsic effect that causes a marked suppression in the true HSC population. Exploring potential mechanisms for a cell extrinsic effect, Mead et al. (2017) noted decreased numbers of endothelial cells (ECs) and mesenchymal stem cells (MSCs) in the FLT3-ITD marrow. The MSCs and ECs from FLT3-ITD mice displayed differences in mRNA expression profiles from WT cells. Based on the profiles, the authors hypothesized that tumor necrosis factor α, which was overexpressed in the niche cells of FLT3-ITD marrow, could have contributed to the niche suppression. Although this proposed mechanism clearly needs more experimental proof, it is intriguing because it offers an explanation for the suppression of normal hematopoiesis that accompanies the disease state of AML. An illustration outlining the proposed model is shown in our figure.

It's still not entirely clear who is correct here. Mead et al. (2017) make a sound argument for their case, but they are relying on a relatively new and delicate technology: single-cell mRNA analysis. And so we return to our philosophical conundrum. Unlike the ancient theological puzzle of angels on the head of a pin, which represents a debate over the trivial, this issue of how many mutant FLT3 receptors are dancing on the surface of the stem cell is a vitally important one. FLT3 inhibitors are moving rapidly into the clinic, and they appear to be effective in improving outcomes for AML patients with FLT3-ITD mutations. If the stem cell giving rise to the disease lacks expression of the mutant receptor, it means FLT3 inhibitors can have no effect on them. In this case, if chemotherapy is unable to eliminate the leukemic stem cell, then certainly chemotherapy plus a FLT3 inhibitor will fare no better, and our most reliable option for cure would remain allogeneic transplant, in which we switch the patient's stem cells for someone else's.



Proposed model of FLT3-ITD expression in HSCs. Cells that have transitioned to MPP cells begin to express the FLT3-ITD mRNA and receptor, but some of them still phenotypically overlap with the true HSCs within the LSK/$CD150^+$/$CD48^-$ population. Expression of FLT3-ITD drives expansion of the MPP cells, which in turn exert a negative effect on the bone marrow niche cells.

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