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A vicious interplay between genetic and environmental insults in the etiology of blood cancers

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Abstract

Over the years, the etiology of cancer has been attributed alternatively to genetic and environmental insults. According to the genetic hypothesis, cancer cells arise as a result of the acquisition of mutations that dysregulate intrinsic mechanisms controlling normal cell growth and survival. In contrast, the environmental hypothesis holds that cancer can be caused by multiple extrinsic challenges that alter normal tissue homeostasis, but may not necessarily involve mutations. It is, however, quite possible that these two mechanisms are not mutually exclusive. According to this third hypothesis, environmental challenges, by mechanisms still poorly understood, may act by imposing a selection pressure that confers a proliferative advantage on cells carrying specific driver mutations, leading to disease initiation. A brief report published in this issue of the journal tested this third hypothesis experimentally and provides new evidence that chronic inflammation, by increasing TNF- α , may positively select malignant hematopoietic stem cells (HSCs) carrying loss-of-function *TET2* mutations that switch the TNF- α signaling responses to activate proliferation rather than inducing quiescence. Furthermore, these mutations skew hematopoietic differentiation toward the myelo-monocytic lineage and the output of macrophages producing TNF- α constitutively, promoting further environment-independent expansion of the malignant HSCs. These findings support a model in which the formation of a malignant population is triggered by a vicious interplay between genetic (*TET2* mutations) and environmental (inflammation) insults suggesting the need for strategies that target both the malignant cells and their environment.

Graphical Abstract

Prince Charming enchanted by the wicked step-mother. This figure summarizes the vicious interplay between genetic (*Tet2* haplo-insufficiency) and environmental (inflammation) insults leading to clonal hematopoiesis and eventually to development of blood cancers. According to (ref), inflammation activates macrophages (Prince Charming) to produce TNF- α , a growth factor which induce healthy HSC into quiescence (sleeping Snowwhite). A HSC clone that would become *Tet2* haplo-insufficient (the awoken *Tet*^{neg} step-mother) would instead be induced by the mutation to proliferate in response to TNF- α acquiring a proliferative advantage over healthy

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HSC. Furthermore, the awoken Tet^{neg} HSC would generate macrophages (the enchanted Prince Charming) producing TNF- α constitutively, leading to further expansion of wicked HSC and possibly to progression to chronic myelomonocytic leukemia (CMML) (original drawing by Roberto di Rocco, Bologna, Italy).

Keywords

Hematopoietic stem cells; blood cancers; driver mutations; environment; microenvironment

Introduction

Experimental evidence in mice and clinical observations in humans indicate that aging is associated with changes in numbers and properties of cells that make up the hematopoietic stem cell (HSC) compartment. These include increases in number and appearance of clones with intrinsically altered programs that reduce their self-renewal and reconstitution activity in transplanted recipients and display restricting lymphoid differentiation thus appearing to favor an output of myeloid cells (1). These changes in aging HSCs is an active area of research with numerous reviews published on this subject in the last 2 years. The evolution of the HSC compartment that occurs with age also has important clinical implications for the development of both malignant and non-malignant human diseases, the incidence of which in humans has age as one of their major prognostic factors (2). However, thus far, this process has been mechanistically analyzed mostly in mouse models. A study published in this issue summarizes results of barcoding experiments indicating that, in mice, age-related changes in the HSC compartment are primarily associated with an appearance and selection of clones carrying deregulated intrinsic programs (3). This paper also discusses data supporting the hypothesis that in mice, age-related HSC changes are driven mostly by epigenetic events that do not alter the cellular genome. In fact, previous parabiosis experiments had indicated that the HSC of old mice could be rejuvenated by exposure to the environment of young animals (4). New observations expand this concept by indicating that HSCs from adult mice obtained by injecting blastocysts with induced pluripotent stem cells (iPSC) obtained from old HSCs have proliferation and differentiation potentials similar to those of young HSCs (5). Therefore, at least in mice, the microenvironment of the blastocyst may rejuvenate old HSC-derived HSCs. Of interest, the microenvironment of the blastocyst had been already demonstrated to be capable of resetting the epigenome of human adult HSCs making them capable of generating erythroid cells expressing embryonic globin genes (6).

In humans, changes occurring in the HSC compartment with age are more complex than in inbred mice because of the longer life span of humans (>70 vs only 2 years), their greater genome diversity and their variable exposure to different environmental insults compared to an animal facility. All these variables have the potential to increase the accumulation of both acquired mutations and epigenetic alterations (2), thus limiting the extrapolation of results obtained on rejuvenation of old HSCs in mouse models to humans. In addition, the profound changes observed in the cellular composition of the bone marrow microenvironment of old individuals suggest that, in humans, microenvironmental cues may also contribute to skewing the myeloid differentiation potential of HSCs associated with aging (2,7). Recognition that the microenvironment is sensitive to external challenges suggests that the environment, by altering the microenvironment, may in turn indirectly affect the number and quality of HSCs. This perspective discusses the possible role played by the environment in the selection of specific HSC clones with age and its implication for the initiation of blood cancers.

Insurgency and selection of HSC clones during development and aging

During the development of all vertebrates, HSC are specified from few common ancestry cells when definitive hematopoiesis is initiated (8). Additional mechanisms contribute to generate further genetic divergence among HSCs at several points throughout ontogeny. In fact, in both mice and humans, the appearance of definitive HSCs is preceded by an active phase of chromosome imprinting, which includes methylation, histone modifications, antisense non-coding RNA-mediated silencing, long-range chromatin interactions and microRNA expression that inactivates expression from one of the two alleles of multiple genes (9). The first and best studied of these events is lyonization, the process that suppresses the transcriptional activity of one of the two X chromosomes to balance the information from this chromosome in female cells thereafter (10). Female HSCs are, therefore, a mosaic of at least two clones, one with an active paternal and another one with an active maternal X chromosome. Since X chromosome inactivation is a random process, at birth the two clones equally contribute to hematopoiesis as documented by the fact that young females heterozygous for the X-linked glucose-6-phosphate dehydrogenase (*G6PD*) gene express proteins from both alleles with the predicted 50:50 ratio (11–12). However, later, hematopoietic cells from these same females often express only one of two alleles, indicating that HSC clones in which the other allele was active had been lost or suppressed (11–12). Interestingly analyses of *G6PD* expression in heterozygous females has also provided the first evidence that blood cancers, and in particular the myeloproliferative neoplasm Polycythemia Vera (PV), originate from a single HSC (13).

In addition to X-linked genes, genetic imprinting suppresses the expression of at least 200–300 tissue-specific autosomic genes (9). Since the alleles most frequently inactivated are the paternal ones, this process has been defined maternal imprinting although in some cases inactivation may involve the maternal alleles (9). Overall, given the high level of allele polymorphisms in the human population, genetic imprinting has the same effects of the “parental disomy” discussed below and leads to the generation of greater numbers of HSC clones in man than in experimental mouse strains.

The generation of novel HSC clones continues after birth as shown by tracking of mutations. The genomic allele polymorphisms of the human population also allows for the continuous production of novel adult HSC clones through the mechanism of chromosome crossing over, i.e. exchange of genetic material between identical chromosomes, during cytodieresis. In fact, in addition to meiosis, chromosomal crossing over may also occur randomly during mitosis leading to the generation of cells carrying only one of two inherited alleles, a genetic phenomenon defined as uniparental disomy (14). Although uniparental disomy should be a rare event, its increasing prevalence indicates that uniparental disomy occurs at frequencies greater than expected in the HSC compartment where it contributes to generate novel HSC clones with age. Because the process of uniparental disomy leads to loss of heterozygosity and may impair the function of anti-oncogenes, it may represent one of the first events in the development of blood cancers by rendering new HSC clones more susceptible to developing driver mutations. This hypothesis was first suggested when uniparental disomy in the region of chromosome 9p that includes the *JAK2* gene was discovered to be a frequent event in PV (15). In this case, uniparental disomy may initiate disease progression by inducing loss of heterozygosity for a polymorphism upstream of *JAK2* and thus increasing the chance of developing the *JAK2V617F* mutation, which is a recognized driver of the disease (16–18). More recently, uniparental disomy for chromosome 4q24 where *TET2* is located has been described to be associated with other myeloid neoplasms, leading to the discovery of loss-of-function mutation of this gene in these disorders (19).

Spontaneous DNA mutations constitute additional genetic insults that may contribute to the generation of new HSC clones with age and which, because of the longer lifespan of humans, occur more frequently in them than in mice (20). The rate of spontaneous mutations has been calculated to be one bp per 1 million bps per cell division. Although HSC activate the DNA repair machinery when re-entering into cycle (21), some of these mutations escape repair generating new HSC clones. Fortunately, these mutations occur mostly in non-coding regions of the DNA or are so disruptive of the biology of the cells that they are lethal. In some cases, however, the mutated HSC clones are viable and may even become the predominant HSC population with age accumulating further driver mutations that transform them into leukemic stem cells (20). In fact, in humans, the risk of developing some blood cancers is strongly predicted by the antecedent acquisition of clonally biased hematopoiesis (22–24).

Not only do uniparental chromosomal disomy and DNA mutations accumulate with age in the HSC compartment with a frequency greater than expected, they also involve limited numbers (10–20 at most) of genes each one of which is associated with a specific blood cancer phenotype. This specificity speaks against a “random” process and predicts the existence of selection pathways, still to be discovered, that favor the insurgency of HSC clones with driver mutations for specific disease phenotypes.

Epidemiological evidence that environmental insults increase the incidence of specific blood cancers

The frequent reports of clusters of specific forms of cancers among non-consanguine individuals who live in restricted geographical areas provides strong epidemiological support for the hypothesis that environmental insults determine the insurgency of specific forms of cancers. One of the clearest supports for this hypothesis is represented by the strong correlation existing between exposure to an environment rich in asbestos and development of mesothelioma (25). However, with few exceptions, the mechanisms which determine the appearance of cancer clusters are seldom investigated due to difficulties in elucidating their origins in sufficiently defined models. Such clusters are thus often dismissed as being caused primarily, if not exclusively, by genotoxic effects of the environment. However, a caveat of this hypothesis is that genotoxic effects are usually random and do not target specific DNA regions.

In the case of blood cancers, a multicenter study in 2008 identified a statistically significant ($p < 0.001$) cluster that included 15 non-consanguineal cases of PV carrying the *JAK2V617F* mutation at the nexus of Carbon, Luzerne, and Schuylkill counties in northeast Pennsylvania (26). The incidence of PV in this very limited area was 4.3 times greater than in the rest of the study area. Although the area of the cluster contained numerous sources of potential genotoxic material, including waste-coal power plants and U.S. Environmental Protection Agency Superfund sites, it was difficult to envision how, in this case, a genotoxic insult alone would have selectively resulted in *JAK2V617F*-driven PV.

Clues that environmental insults may drive the development of blood cancers by activating the stress pathway that, in turn, alter a normally supportive microenvironment have recently been reported. These were provided by observations that mice subjected to a chronic erythroid stress experience sustained activation of the thrombopoietin/MPL/JAK2 pathway similar to that induced by the *JAK2V617F* mutation and with age developed myelofibrosis, a myeloproliferative disease closely related to PV (27). In addition to chronic erythroid stress, the development of myelofibrosis in this model was dependent on the generation of disease-specific megakaryocytes expressing high levels of TGF- β . This, in turn, induced a microenvironment supportive of the malignant HSCs (28). In fact, while high levels of TGF- β have been found to drive healthy HSCs into quiescence (29), as part of the transformation process, malignant HSCs became insensitive to TGF- β and continued to proliferate as long as the TGF- β -induced microenvironment was there to support them (30). These data started to delineate a vicious cycle between an environmental insult (induction of erythroid stress), a specific driver mutation (activation of the TPO/JAK2 axis) and microenvironmental cues (TGF- β) in the initiation of a specific blood cancer (myelofibrosis). In the case of the PV cluster in Pennsylvania, it may be argued that the toxic waste may have induced an analogous chronic erythroid stress response to an oxidative insult that reduced the half-life of the red blood cells. However, experimental evidence that continuous exposure to toxic waste triggers chronic erythroid stress is scanty.

New evidence for the existence of a vicious circle between genetic and environment insults in the initiation of blood cancers.

TET2, the gene that encodes Tet methylcytosine dioxygenase 2 is located in the region of human chromosome 4 at q24 and is frequently involved in microdeletions and/or uniparental disomy leading to loss of heterozygosity in patients with diverse myeloid malignancies, including myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPN), chronic myelomonocytic leukaemia (CMML) and primary and secondary acute myeloid leukaemias (AML) (19). *TET2* encodes a protein that catalyzes removal of the methyl group present on the fifth carbon on the cytosine base of multiple genes making them prone to activation (31). Loss of *TET2* function thus impairs the ability of cells to activate affected genes, including those necessary to suppress the inflammatory response (32). HSCs from Tet2-deficient mice show an accelerated appearance of several features of old HSCs, including a restricted output of myelo-monocytic cells. Moreover, with age these mice develop a phenotype reminiscent of human MDS and CMML (cytopenia, monocytosis, splenomegaly) (33). The skewed myelo-monocytic differentiation of Tet2-deficient HSCs includes an ability to generate macrophages which produce high levels of TNF- α constitutively (34).

The brief report published in this issue of the journal (35) presents data from *in vitro* studies suggesting that TNF- α stimulates Tet2-deficient HSCs to produce an increased number of myeloid colonies in semisolid media. In contrast, TNF- α reduced the number of colonies produced *in vitro* by healthy HSCs as expected from the well described ability of TNF- α to induce normal HSC quiescence (36). These results provide circumstantial evidence that a TNF- α -rich microenvironment sustained by the macrophage progeny of Tet2-deficient HSCs provides a proliferative advantage to malignant HSCs over their healthy counterparts, leading to disease progression. The etiology of CMML may thus be similar to that of myelofibrosis since, as discussed by a recent review by the Green laboratory (37), in both cases the progeny of malignant HSCs are responsible for creating a microenvironment that selectively sustains their proliferation. In this regard, it is interesting to speculate that ascorbic acid supplementation, in addition to prevent disease progression in these Tet2-deficient mouse models by phenocopying Tet2 functions (38,39), may be efficacious also for its well-known anti-inflammatory effects (40) (or *vice versa*, its anti-inflammatory effects are partially mediated by its effects on *TET2* activity). Furthermore, these observations suggest that environmentally sustained inflammation may also determine the great prevalence of *TET2* mutations, the appearance of which often precede that of the driver mutations, found in patients with myeloproliferative neoplasms (41) since, as *Tet2*-deficient progenitor cells, also *JAK2V617F*-progenitor cells from patients and mouse models proliferate *in vitro* in response of TNF- α (42).

The paper by Gong et al also published in this issue of Experimental Hematology provides *in vitro* evidence that TGF- β produced by megakaryocytes is responsible to suppress normal HSC in acute myeloid leukemia (43) and suggests that suppression of normal hematopoiesis by inflammatory molecules produced by the microenvironment may be a general mechanism in the disease progression of blood cancers in humans

The results described by Abegunde et al (34) also provide insights into the mechanism that may be critical for disease initiation. In fact, the numerous environmental insults known to activate TNF- α production by normal macrophages and produce a prolonged inflammatory state with sustained increases in local TNF- α levels, may be sufficient to support the survival/proliferation signals necessary to initiate disease from latent or later appearing Tet2-deficient HSCs. This concept is attractive because it is also testable in animal models.

Conclusions and Future perspective

In addition to their genotoxic effects, environment insults may contribute to cancer initiation by establishing stress conditions that provide a proliferative advantage to HSCs carrying specific driver mutations. Accordingly, appropriate pharmacological control of inflammation may play an important role in preventing the development of various types of cancers. Further understanding of the mediators of inflammation that may contribute to the establishment of such a “pro-cancer microenvironment” may also aid in the design of more effective therapies that target both the malignant HSCs and the altered microenvironments that favour their growth. Last, but not least, this hypothesis should stimulate the design of further experiments to examine the nature and significance of potentially other similar cancer-promoting circumstances that determine outcomes in cells already carrying independent oncogenic mutations.

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