Somatic mutations and the hierarchy of hematopoiesis

Ripples from mutations in hematopoiesis

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Clonal disease is often regarded as almost synonymous with cancer. However, it is becoming increasingly clear that our bodies harbor numerous mutant clones that are not tumors, and mostly give rise to no disease at all. Here we discuss three somatic mutations arising within the hematopoietic system: *BCR-ABL*, characteristic of chronic myeloid leukemia; mutations of the *PIG-A* gene, characteristic of paroxysmal nocturnal hemoglobinuria; the V617F mutation in the *JAK2* gene, characteristic of myeloproliferative diseases. The population frequencies of these three blood disorders fit well with a hierarchical model of hematopoiesis. The fate of any mutant clone will depend on the target cell and on the fitness advantage, if any, that the mutation confers on the cell. In general, we can expect that only a mutation in a hematopoietic stem cell will give long-term disease; the same mutation taking place in a cell located more downstream may produce just a ripple in the hematopoietic ocean.

Keywords:
clonal evolution; hematopoietic stem cells; mutations; progenitor cells; stochastic dynamics

Introduction

The machinery for DNA replication in eukaryotic cells is very sophisticated and highly accurate; however, like all physical machines, it is not perfect. The average error rate, *i.e.* the spontaneous mutation rate, is around 1×10^{-9} /base/replication [1] or $\sim 1 \times 10^{-7}$/gene/replication [2, 3]. Additional mutations can result from environmental genotoxic agents (radiation, chemicals including therapeutic agents, and viruses). Given that our genome is $\sim 3 \times 10^{9}$ base pairs in length, and given that our body consists of approximately 10^{14} cells, we must admit we are riddled with mutations; indeed, there is probably at least one cell harboring any one possible mutation in our body. However, the phenotypic effect of a mutation depends on the DNA sequence context as well as on the cell context: thus, many mutations are silent or neutral in any cell; and cells that do not express the mutant gene cannot be affected by any mutation in that gene.

Acquired mutations – also called somatic mutations, to distinguish them from mutations that are inherited through the germ cell line – can produce clones of mutant cells in our bodies, and they are at the root of neoplastic transformation [4–8]. Serial accumulation of mutations can produce a cell that ignores regulatory growth control mechanisms and ultimately may give it the ability to invade other tissues [5, 6].

Mutant clones are so strongly associated with the development of tumors that the two phrases are often regarded almost

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Abbreviations:
CML, chronic myeloid leukemia; **CFU-GEMM,** granulocyte, erythroid, megakaryocyte and monocyte colony-forming unit; **CFU-GM**, granulocyte and monocyte colony-forming unit; **HSC,** hematopoietic stem cell; **MPD,** myeloproliferative disease; **PNH,** paroxysmal nocturnal hemoglobinuria.

as synonymous with each other. However, here we will briefly review evidence that many clones, even when harboring potentially dangerous mutations, may never become tumors. Indeed, thanks to powerful contemporary technologies such as flow cytometry, polymerase chain reaction, and increasingly deeper genome sequencing, such mutant clones are being increasingly identified in healthy individuals. We explore this notion with reference to the hematopoietic system for two reasons: (a) we have a reasonable model of the dynamics and of the hierarchy of hematopoiesis; (b) we have three examples of extensively investigated somatic mutations specifically associated with serious blood disorders: (i) the Philadelphia translocation producing the *BCR-ABL* fusion gene characteristic of chronic myeloid leukemia (CML) [9]; (ii) the inactivating mutations of the *PIG-A* gene characteristic of paroxysmal nocturnal hemoglobinuria (PNH) [10], and (iii) the gain of function mutation V617F of the *JAK2* gene characteristic of myeloproliferative disorders (MPD) [11–13]. In all three cases the same mutations can be seen in the absence of disease, and what we learn in understanding how this happens might be applicable as well to other systems in the body.

Hematopoiesis has many cells and many cell types

Blood cells have a high rate of turnover: therefore, hematopoiesis is a massive operation, with a product of $\sim 3 \times 10^{11}$ cells per day; it is also highly regulated, as it is responsible for maintaining blood cell homeostasis. The majority of the cells produced belong to the erythroid lineage (they are reticulocytes that will mature in the circulation to erythrocytes, or red blood cells); however, granulocytes have an even faster turnover since they last in the circulation only a few hours [14–16].

Hematopoiesis is maintained by a pool of hematopoietic stem cells (HSC) that, by definition, are able to both self-renew and give rise to progeny cells that can differentiate along various lineages, giving all types of blood cells [17]. Experimental and theoretical considerations suggest that only a fraction of HSC actively contribute to hematopoiesis: in humans the best estimate is of the order of 400 cells [18, 19] and they each divide approximately once per year [19, 20]. Linking the HSC and mature blood cells is a hierarchically organized process where cells divide and become increasingly differentiated (Fig. 1).

We have recently proposed a mathematical formulation of this process [21], where cell renewal and differentiation are stochastic and coupled processes [22, 23]. From this model we can work out the dynamics of hematopoiesis. This in turn makes it possible (i) to infer the site of origin of any mutant clone when we know its size, and (ii) to estimate the time during which a clone with a neutral mutation will remain in the circulation [24–26] (this will be a lower limit: clones with mutations that provide a fitness advantage will be expected to survive for longer [24, 25]).

A very important consequence of this architecture is that it reduces the probability that mutant clones dominate hematopoiesis, reducing the risk of cancer [4]. This is because once a mutation occurs in a normal hematopoietic cell, an evolutionary race between normal and mutant cells is set in motion. This evolutionary dynamical competition [4, 7, 8] may or may not proceed all the way to the development of a myeloid neoplasm [27, 28], depending on whether mutated cells exhibit any (fitness) advantage compared to normal cells. For instance, whenever mutation(s) increase the self-renewal probability of progenitor cells, the resulting fitness advantage may enable the clone to expand, leading to disease [25]. On the other hand, the stochastic nature of hematopoiesis means that clonal extinction is also possible (albeit with a small probability) even for mutant cells with a fitness advantage compared to normal cells [29]. We now use this model to illustrate the inevitable presence of small clones with well-known genetic defects in the circulation of otherwise healthy adults.

Normal mutation rate — Still many mutations

Whereas the size of the active HSC pool is small, the number of progenitor cells such as granulocyte, erythroid, monocyte, and megakaryocyte colony-forming units (CFU-GEMM) and granulocyte and monocyte colony-forming unit (CFU-GM) is much larger. There are approximately $\sim 1.0 \times 10^5$ CFU-GEMM cells [30] and significantly more CFU-GM cells ($\sim 10^8$). We estimate that each CFU-GEMM may contribute to hematopoiesis for an average of $\sim$60 days (range 40–340 days), and that it replicates at an average rate of once every 50 days (range 35–285 days), while the replication rate of CFU-GM is significantly faster [21]. Therefore, even at a normal mutation rate, many cells with specific defects will appear. However, it is important to remember that (i) the net progeny of each of these cells is small, and (ii) the average time they contribute to hematopoiesis also decreases quickly as they are more mature. Since mutations can occur in any cell that is dividing, the majority of mutations will arise in the larger populations of cells at the later stages of hematopoiesis and these will have very little clinical significance: indeed, in general the resulting clones will be not only small in size (often below current detection limits), but also short lived, or transient, as they will be quickly washed out of hematopoiesis. Consequently, the mutations that really matter are those within the HSC, because they might produce a disease, whereas mutations in more mature cells will cause only inconsequential ripples in hematopoiesis. The architecture of epithelia (*e.g.* a colonic crypt, where hierarchy is directly reflected in topography) has similar implications [31, 32], supporting the general applicability of the concepts developed here.

Cells with the Philadelphia chromosome

Fusion genes resulting from chromosomal translocations are among the most powerful oncogenic mutations. The incidence of any chromosomal abnormality is around 1.0×10^{-5} per cell per replication [33]; however, the probability of a specific chromosomal translocation is of course much lower [34, 35]. CML is characterized by the Philadelphia chromosome

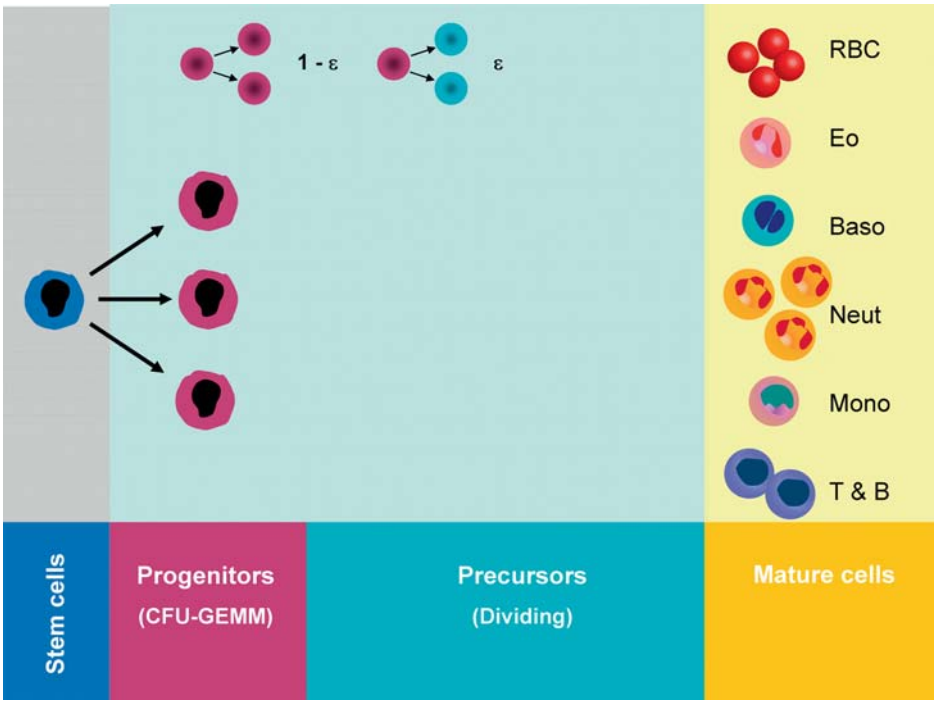

Figure 1. Schematic representation of hematopoiesis. Mature blood cells undergo continuous turnover and are replaced by cells produced in the bone marrow. At the root of the process are the hematopoietic stem cells (HSC) that generate all other blood cell lineages. Progenitor and more mature cells are selected stochastically to divide. With a probability ε, the daughter cells differentiate, while with probability 1-ε, the cell self-renews and both daughter cells remain in the same stage of differentiation. Cells in a given stage j replicate at a rate r_j while the rate of replication between successive differentiation stages is constant, $r_j/r_{j-1} = r$. Similar to age-structured population models, one can separate different stages of cell maturation into different compartments, such that cells lost from compartment j due to differentiation are replaced by input from compartment $j-1$, all the way back to the HSC. This coupling of adjacent compartments ensures homeostasis. For normal hematopoiesis $\varepsilon \approx 0.85$, meaning that cells tend to differentiate rather than self-renew (for details, see ref. 21). Within such a multi-compartment model, stem cells occupy the root compartment 0; CFU-GEMM cells extend approximately till compartment 8, whereas precursor cells diversify along the hematopoietic tree through the remaining ~23 compartments before leaving the bone marrow. The widely accepted model illustrated in this cartoon (Fig. 1) is certainly an approximation, especially because it suggests a sharp definitive demarcation between say, a stem cell and a progenitor cell. In fact, it is possible to envisage a stem cell that is still capable of self-replication and is still multipotent (the two defining properties of stem cells), but is much more prone to become a myeloid or an erythroid cell rather than a lymphoid cell. Such a cell would have to be regarded as somewhere between a stem cell and a CFU-GEMM.

[t(9;22)(q34;q11)] [36] that produces the *BCR-ABL* fusion gene. How often will this abnormality appear in HSC? For a population of adult humans that live up to 70 years of age, we can estimate the average cumulative probability of having an HSC with the Philadelphia chromosome (P_{ph}) as: $P_{Ph} = N_{SC} \cdot R \cdot \mu_T \cdot \mu_9 \cdot \mu_{22}$, where $N_{SC} \sim 400$ is the number of active HSC [18, 19], $R = 70$ refers to the average number of divisions of each HSC during the lifespan of the organism [19, 20], $\mu_T = 10^{-5}$ is the probability of any chromosomal translocation per cell per replication, $\mu_9 = 0.011$ is the frequency of chromosome 9 breaks at band q3 [34] and $\mu_{22} = 0.0056$ is the frequency of chromosome 22 breaks at band q1[34]. The result leads to an incidence of 1.7:100 000 individuals, very close to the reported incidence of the disease in the United States (1.5 per 100 000 per year, SEER database and [37]). This assumes that one or a few leukemic stem cells are enough to drive the chronic phase of CML [27, 28, 38], consistent with experimental observations that *BCR-ABL* expression in a single HSC can lead to chronic-phase CML in animal models of the disease [39].

The same translocation can also occur in CFU-GEMM cells. Given that an average human adult has $\sim 10^5$ CFU-GEMM, which on average replicate approximately seven times per year, we estimate a 50-fold increase in incidence, *i.e.* 1:2300 healthy adults will have such a CFU-GEMM cell with the Ph' chromosome. If we consider mutations in more mature cells, the incidence will be even higher. However, as detailed before, we expect such a more frequent clone to expand transiently and then disappear – a ripple in hematopoiesis. This provides at least one simple explanation of why healthy people can have detectable Ph'-positive clones but no disease [9].

Cells with the PNH phenotype

Flow cytometry can identify in every healthy adult [10] very rare circulating populations of neutrophils and erythrocytes that lack glycosylphosphatidyl inositol linked (GPI-) proteins on their surface (*e.g.* CD55 and CD59). This phenotype is due to acquired somatic mutations in the *PIG-A* gene that lies on the X-chromosome and is critical for synthesis of the GPI anchor

[40]. Many extracellular proteins are bound to the phospholipid bilayer through a post-translational modification that links them to a GPI anchor. Thus, a single mutation in one gene can alter the fate of many cell surface proteins that share this common anchor and give rise to the PNH phenotype. Both CD55 and CD59 are required for protection of red blood cells from complement-mediated attack. In the absence of the proteins, red blood cells undergo intravascular hemolysis leading to hemoglobinuria – hence the name of the disease. Unlike *BCR-ABL* and *JAK2V617F* (see below), each of which is a unique gain of function mutation, these *PIG-A* mutations entail loss of function (the function being GPI synthesis). Therefore, their spectrum is quite heterogeneous [10, 41] (nearly every patient has a personal one), as it is much easier to break something delicate than to make a superior product!

GPI- red cells and neutrophils might arise from a single mutation in a common progenitor (in this case, for instance, a CFU-GEMM), or from two independent mutations in cells committed to either neutrophil or erythrocyte differentiation (Fig. 2) [24, 25]. Given that the mutation rate in *PIG-A* is normal ($\sim 5 \times 10^{-7} - 30 \times 10^{-7}$ per replicating cell) [3, 42], the probability that in any given period there is a CFU-GEMM cell with a mutation in *PIG-A* is given by the product of the average number of CFU-GEMM cell replications in this time interval and the mutation rate for *PIG-A*: effectively a probability of 0.9 ($30 \times 10^{-7} \times 3 \times 10^{5}$) [10]. Moreover, since CFU-GEMM cells contribute to hematopoiesis for a finite time interval, such a clone is expected to disappear in time, although it may be promptly replaced by a new GPI- clone. Therefore, most adults will have transient clones of *PIG-A* mutated cells: another ripple in hematopoiesis. In contrast, mutations in *PIG-A* would be significantly less common in HSC. However, in this case the mutant clone can possibly expand and lead to disease (PNH), either due to neutral drift [26] or as a result of a fitness advantage imposed by the local microenvironment [40, 43]. We have shown previously [26] that, even without selection, the population frequency of PNH in the US is not far from what our model would predict.

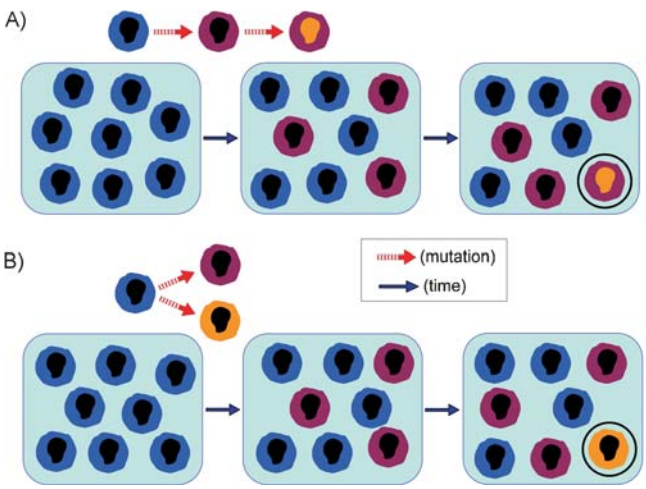

Figure 2. Multiple mutations in cell populations. Once a mutant clone has formed, its cells can acquire additional mutations. The probability that this happens depends on the number of cells at risk, which in turns depends on the fitness of the mutant clone. From our mathematical model we infer that when we find two different mutations, it is not very likely that one has followed the other (model A); it is more likely that a new cell has arisen through a second independent mutation (model B).

*JAK2*V617F in health and disease

Many patients with a chronic myeloproliferative disease (MPD) have an activating mutation in *JAK2* (*JAK2*V617F) [11–13]. The *JAK2* coding DNA is 5285 base pairs in length and a single base substitution at position 617 converts a valine (V) codon to a phenylalanine (F) codon: only a guanosine to uracil substitution (G→U) in the first position of the codon can effect this amino acid substitution. The probability that this specific base is mutated is 1×10^{-9}, and the probability of the specific U for G replacement is 1/3. Therefore, the overall probability that such a specific mutation occurs is $\sim 3 \times 10^{-10}$ per replication. It has been shown in animal models that the *JAK2*V617F mutation in an HSC gives a phenotype similar to polycythemia vera, sometimes with bone marrow fibrosis [44, 45]. The chance that this specific mutation occurs at least once within the HSC pool in a typical human lifetime is given by $400 \times 70 \times 3 \times 10^{-10}$. Therefore, the lifetime incidence of this mutation in an HSC is approximately 1:100 000, which again is very close to the actual incidence of PV in the general population [46]. It is expected that the *JAK2*V617F mutation confers a fitness advantage onto the mutated HSC – enabling it to expand gradually into a large clone [47] – since that cell will have become independent of growth factor stimulation. Given that the rate-limiting step is the appearance of the first mutant cell [48], it is not surprising that our estimate above is quite close to the prevalence of clinical disease.

In a recent analysis of an otherwise un-selected population of hospitalized patients, the *JAK2*V617F was found in 0.94% of cases [49]. The vast majority had normal blood counts and a very low allele burden (<5%). A similar finding was reported in blood donors with a high hematocrit [50]. An obvious question is how to explain the presence of a pathogenic mutation in people who do not have the respective pathology. One possibility is of course that some of these subjects are in a prodromal stage of MPD; however, this cannot apply to all of them, or the prevalence of MPD would be much higher than it actually is. Another possibility is that the mutation has taken place not in an HSC, but in a CFU-GEMM cell (just as we have suggested for *BCR-ABL* and for *PIG-A* mutations). In the absence of a fitness advantage, the most likely scenario will be for the clone to remain detectable for around a year and eventually disappear without leading to any MPD. However, we do not know yet the time course of the *JAK2*V617F clones in these subjects who do not have MPD. On the other hand, we do know in this case the precise mechanism whereby an HSC having that mutation will have a growth advantage, and it seems reasonable to expect that this advantage will also hold for a CFU-GEMM cell with the same mutation. Therefore, we can suggest another possibility, *i.e.* that this mutation has the ability not only to increase but also to prolong the contribution to hematopoiesis of the CFU-GEMM in which it has taken place (Fig. 3). Thus, when the V617F mutation hits *JAK2* in an HSC, the resulting growth

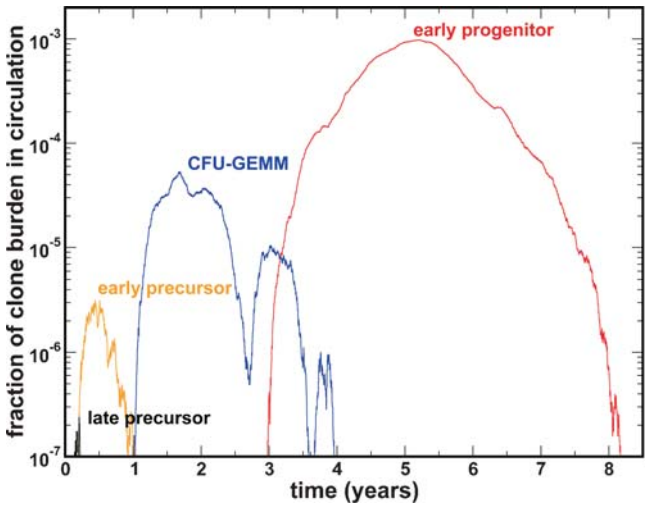

Figure 3. Mutations can occur in any cell within hematopoiesis. However, both the size of the circulating clone and its lifetime depend on the location of the cell of origin. As illustrated in the figure, mutations in more primitive cells give rise to larger clones that survive for longer, taking also a longer time to appear in the circulation. On the contrary, the smaller clones caused by mutations of more differentiated (precursor) cells appear in the circulation much more rapidly after the causal mutation, but they are smaller and survive shorter. In other words, they give rise to a small disturbance of mutant cells progressing throughout hematopoiesis – a ripple.

advantage is so large that the resulting clone becomes predominant, and often takes over hematopoiesis; when the same mutation hits a CFU-GM it will give just a tiny ripple; when it hits a CFU-GEMM the ripple is significantly larger (Fig. 3). Although at the moment this hypothesis is entirely speculative, it could help to explain not only the findings just mentioned, but also why the clinical manifestations of this unique mutation are so variable. Given that the impact of a mutation is cell context dependent, it is also possible that the same mutation that arises within different subpopulations of primitive hematopoietic cells can lead to distinct disease phenotypes such as essential thrombocythemia or idiopathic myelofibrosis, since the latter two disorders are often associated with the *JAK2*V617F mutation [11–13]. However, a substantial fraction of patients with essential thrombocythemia or idiopathic myelofibrosis do not have the *JAK2*V617F mutation. It is possible that in these patients, another, yet-to-be-discovered mutation downstream of *JAK2* but in the same signaling pathway is present and can explain the phenotype. In our analysis we do not specifically consider the possibility that a primitive hematopoietic cell (*e.g.* CFU-GM) acquires stem cell line properties due to the acquisition of specific mutations [51–53]. However, if such a mutation were to occur, we expect the fate of that cell to be similar to that of a hematopoietic stem cell and the same reasoning as discussed previously should apply.

Conclusions

An understanding of the structure and dynamics of cell populations at risk of mutations can provide detailed explanations for many phenomena observed (and yet to be observed) in both health and disease. Working out the dynamics of this system from clinical data alone is difficult, because in each patient there are bound to be complicating factors, and because any treatment received by the patient will affect the dynamics. On the other hand, mathematical analysis based on what we know of the hierarchical structure of hematopoiesis can help to understand the incidence and dynamics of mutant clones, which can then be validated by clinical studies. Within such a (large) population as the blood-forming bone marrow the occurrence of mutations is inevitable, but most of them will simply have no consequences – in effect they create small ripples in an ocean of cells. To be aware of these concepts is even more important today than it has been before, as new technologies improve our ability to identify small mutant populations in humans: we must refrain from automatically diagnosing them with a disease. Rather, we should arrange a careful follow-up in time (we could call it the clinical dynamics of clones *in vivo*), while avoiding unnecessary treatment, lest the cure is worse than the disease! Since not only the hematopoietic system, but also most other tissues in the body, for instance epithelia, have evolved a similar hierarchical organization, they too behave mathematically as age-structured populations [31, 32, 54]. Therefore, the principles discussed here are probably applicable to virtually every other tissue in the human body and, given the general conservation of tissue architecture across animals, to many other multicellular life forms.

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