SOME DYNAMIC ASPECTS OF HEMATOPOIETIC STEM CELLS

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ABSTRACT

Hematopoiesis is the process responsible for maintaining the number of circulating blood cells that are undergoing continuous turnover. At the root of this process are the hematopoietic stem cells (HSC). In the following we discuss various dynamic aspects of HSC behavior ranging from the number of active stem cells, their expansion during ontogeny and the importance of stochastic effects on their behavior. We show how mathematical modeling of HSC behavior can provide important insights on these cells and clarify the implications of these dynamical aspects on healthy and sick individuals, as such providing rational explanations for relevant clinical observations on disorders that originate in this group of cells.

Introduction

Circulating blood cells have a finite lifespan and are continuously being replaced by new cells produced in the bone marrow. The cellular output from hematopoiesis under equilibrium conditions is staggering and of the order of 3.5×10^{11} cells per day [1]. This number is increased under conditions of higher demand (e.g. bleeding or infection) or as a result of neoplastic transformation of hematopoietic cells as in chronic myeloid leukemia (CML) [2] or polycythemia vera (PV) [3]. At the root of blood formation are the hematopoietic stem cells (HSC) that reside in the bone marrow [4,5]. The presence of these cells was initially inferred from bone marrow reconstitution experiments after total body irradiation in mice [4]. HSC make bone marrow transplantation possible, a procedure that has provided curative therapy for a variety of otherwise lethal genetic/metabolic or neoplastic disorders [6]. HSC can be operationally divided into an active and a reserve pool. Cells in the active pool contribute to hematopoiesis and are associated with the endosteal surface of bone [7]. Osteoblasts on the bone surface seem to play a critical role in forming the so-called stem cell niche, the structure that supposedly harbors stem cell(s) and defines their function [7,8]. Cells in the reserve compartment are inactive and occupy other sites within the bone marrow. The definition of a HSC requires two concomitant properties: self renewal and multipotent differentiation [9]. Self renewal refers to the cell's ability to give rise to progeny that retain the characteristics of the parent cell. The capability of multipotent differentiation means that the progeny cell can ultimately give rise to cells with different functional capabilities.

HSC are believed to be different from all other blood cell types because they stand at the root of the hematopoietic tree, which portrays the hierarchical organization of hematopoiesis (Figure 1) [10], where the more committed and specialized cells reside in branches further away from the root of the tree. In the case of HSC, the progeny cells form the granulocytes (and their subsets), lymphocytes, monocytes, erythrocytes and platelets. The presence of a HSC can only be inferred *a posteriori* from serial transplantation experiments that out of necessity will require a cell to have *both* properties to sustain hematopoiesis. There is increasing evidence that once a HSC is selected to contribute to hematopoiesis, it will do so for a very long time, if not the lifetime of the individual [11]. In the following, we provide a theoretical framework to

estimate the number of active HSC during human ontogeny and in adult life and to demonstrate the importance of stochastic dynamics on the evolution of acquired mutations in such a pool of cells.

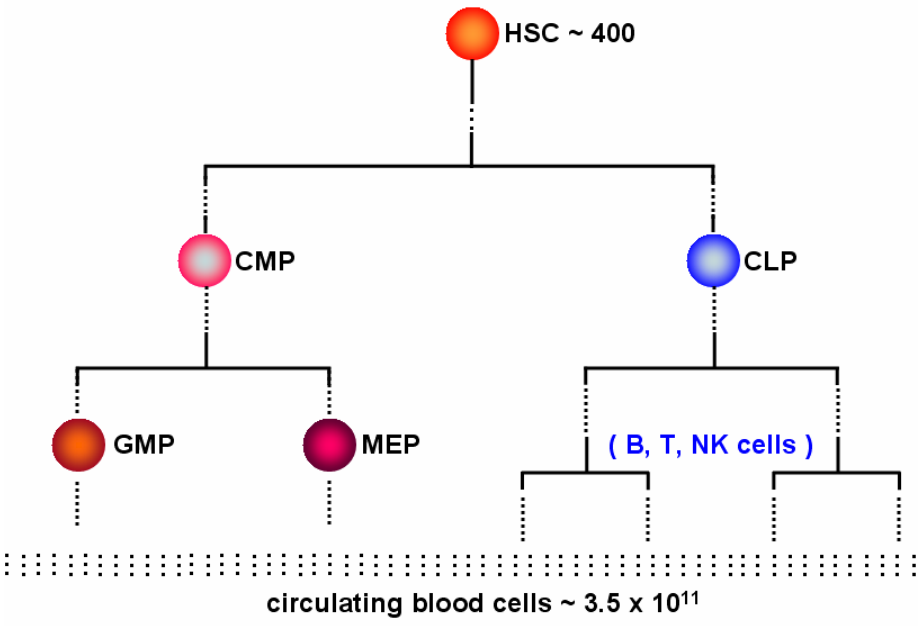

Figure 1. Hierarchical tree of hematopoiesis. Hematopoietic stem cells (HSC) stand at the root of the tree. All other lineages of hematopoietic cells derive from HSC and result from successive branching down the tree as cells become increasingly specialized. Recently, the overall number of major branches in this tree has been estimated to 32. The figure illustrates some of the major branches, giving rise to the myeloid lineage with associated common myeloid progenitors (CMP) and lymphoid lineage with associated common lymphoid progenitors (CLP). The B-, T- and NK-cells arise further down in the tree and derive from CLPs, whereas granulocytes, macrophages, platelets and red blood cells derive from CMPs. (MEP: Megakaryocyte Erythrocyte Precursors; GMP: Granulocyte Macrophage Precursors).

Scaling of the active stem cell pool across mammals

How many stem cells actively contribute to hematopoiesis in a given adult mammal? Until now, no unambiguous answer has been provided in the laboratory.

Studies from patients with chronic granulomatous disease [12] suggest a number around 400, whereas experimental studies in patients subject to bone marrow transplantation indicate that, after the procedure, hematopoiesis is maintained by ~111 stem cells [13]. On the other hand, the demands on hematopoiesis are clearly different across mammals [14]. Indeed, it can be shown that the total marrow output during the lifetime of a mouse is roughly the same as an adult human produces in a day. In order to understand this body of evidence, one may resort to the field of allometry [15] combined with the above

definitions of active and reserve HSC pools. In biological systems, many observables (Y) related typically to nutrient transportation, such as metabolic rate, tree trunk thickness etc often scale with mass as $Y \sim Y_0 M^b$ where b is typically a multiple of ¼. Competing explanations for the origin of these exponents exist [16,17,18]. With respect to HSC and hematopoiesis, we note that (i) hematopoiesis has appeared only once during evolution (the similarities between the process across mammals support this), (ii) although hematopoiesis is distributed across various bones, it is functionally coupled by the circulation and so HSC effectively function collectively, and (iii) from the definition of the HSC, every active HSC is equally represented in the circulation of a given mammal. Under these premises, we were able to provide an allometric estimate of the size of the active stem cell pool in mammals (N_{SC}) as a function of their mass [19]. The best marker of cellular marrow output is the total circulating reticulocyte count (R_T) that can be estimated in many mammals from knowledge of the blood volume, the red cell count and the percentage of reticulocytes present in the circulation. Taking into account the variable maturation rate of reticulocytes across species, we determined that R_T scales with the mass of the adult mammal as $R_T \sim M^{3/4}$ [19]. From (iii) above, we are led to conclude that $N_{SC} \sim M^{3/4}$. Therefore, if N_{SC} and the mass of *any* adult mammal are known, one can determine N_{SC} for *any other* adult species. Using this relationship calibrated for cats allowed us to estimate that in humans $N_{SC} \approx 400$, in agreement with experimental observations [12]. The same relationship suggests that after bone marrow transplantation, a typical adult has $N_{SC} \approx 116$, again in excellent agreement with experimental data [13]. The scaling $N_{SC} \sim M^{3/4}$ allows us to explore the size of the hematopoietic stem cell pool of other mammalian species. Our model predicts that 1 HSC can maintain hematopoiesis in a mouse for its lifetime [19], a prediction that is supported by experimental observations [20]. Naturally, this lower limit extends to the smallest mammal, a shrew with a mass of 3 gram. On the other hand, while there is no experimental validation of the active SC pool in elephants, our scaling predicts that an Asian Elephant (*Elephas maximus*, ~ 4500 kg) has an active stem pool comprising ~9600 cells. Naturally, the species specific HSC replication rate (B) also follows an allometric

relationship with adult mass: HSC replicate at a rate which decreases with increasing mass ($B \sim M^{-1/4}$) [19]. Interestingly, it has been proposed that the total number of hematopoietic stem cells is conserved across mammals [21], the total number lying somewhere between 11000 and 22000. Although there is (albeit limited) evidence that the number of HSC may be constant across mammals ranging from mice, rats to cats and humans and possibly elephants [14,22,23,24], this result apparently contrasts with the allometric considerations above. However, the estimates of $\sim 10^4$ stem cells do not distinguish between the active and the reserve pool. Our allometric estimates concern the active stem cell pool. Hence, it is gratifying that our estimates do not exceed the limits proposed even when extrapolated to the largest terrestrial mammals.

Expansion of the HSC during human ontogeny

The HSC compartment is an important therapeutic target because there are a number of hereditary/congenital HSC and non-HSC disorders that are amenable to gene therapy approaches. Thus, understanding the size of the active HSC pool in newborns and how the active pool increases in time to adult levels is highly relevant. A small active HSC pool will require highly efficient cell transduction for correction of a genetic/metabolic defect. To obtain insights into the size of this pool during human ontogeny, we have applied the previously discussed allometric scaling relating reticulocyte count and mass to data derived from human growth. We found that the active HSC pool in *Homo sapiens* scales linearly with mass. More importantly, the number of active HSC in newborn babies is quite small, in the range of 20 to 40 cells [25]. Assuming a conserved total number of HSC across mammals, it is at present unclear whether babies are born already equipped with the full pool of HSC or whether their population expands in time to reach the estimated adult size.

Stochastic dynamics within the HSC pool

The small number and slow replication rate of active HSC can have serious implications on the evolutionary dynamics of mutations within this cellular compartment. HSC tend to contribute to hematopoiesis for a long time (probably years in humans) and therefore mutations in these cells can be retained forming a clonal population which may expand or decay over time. In the absence of immortal DNA strand co-segregation [26], the two main mechanisms that preclude the frequent appearance and rapid expansion of

mutant cells are (i) the slow replication rate of these cells ([19,27], and (ii) expression of plasma membrane carriers that effectively efflux a broad spectrum of genotoxic agents (e.g. P-glycoprotein)[28]. However, mutations in HSC do occur and are responsible for a number of neoplastic and non-neoplastic disorders such as chronic myeloid leukemia (CML) [29] and paroxysmal nocturnal hemoglobinuria (PNH) [30] respectively. Presumably the number of active HSC is determined by the number of available niches which must correlate with the demands for hematopoietic cell output. HSC are under the influence of various signals from the cells forming the niche, the extracellular stroma, cytokines and growth factors. The overall input from these various sources together with the transcriptome of the cell presumably define the behavior of the HSC and influence whether a given cell replicates, the symmetry of replication and the commitment to differentiation of the progeny cell along a specific pathway. The complexity of the process can be approximated by a stochastic process akin to Markov dynamics. This is supported by experimental evidence that hematopoiesis may be a stochastic process [31,32]. The fact that the number of terminally differentiated blood cells appears fairly constant reflects their sheer numbers at the end of the hematopoietic tree, that ultimately mask the stochastic nature of the dynamics that occur at all levels of hematopoiesis, the impact of which is stronger the closer to the root a cell is (notably stem and progenitor cell levels, see Figure 1) [10,33].

Under steady state conditions, we can consider that HSC are constant in number and behave as a homogenous population due to chemical coupling [34]. The evolutionary dynamics of such a population can be modeled by a simple stochastic birth-death process known as a Moran process [35] (Figure 2A) where in each event one cell is chosen for reproduction and another is chosen for elimination. The rate at which events take place is dictated by the replication rate of HSC as deduced from the allometric relations. Normal cells chosen for reproduction can mutate with probability μ. Experimental observations estimate that the normal mutation probability per gene is of the order of $\mu \approx 10^{-7}$ per replication [36]. Thus, when a normal stem cell is selected to reproduce, with probability $1-\mu$, it gives rise to two normal daughter cells, while with probability μ, a normal cell and a mutated cell are produced (Figure 2B). When mutated HSC reproduce, they give rise to more mutated cells since the probability of a mutant cell to revert to

normal (wild-type) is essentially negligible. Reproduction increases the number of HSC by one and, therefore, one cell from the whole pool is chosen at random for elimination or, perhaps more precisely, for export, as the cell chosen initiates its pathway of increasing specialization down the hematopoietic tree (Figure 1). Cells are chosen for reproduction proportional to their fitness, r. Normal cells have fitness $r = 1$, cells with mutations that enhance their fitness have $r > 1$, while mutations that confer a fitness disadvantage have $r < 1$. Figure 2 illustrates the process of stem cell replication.

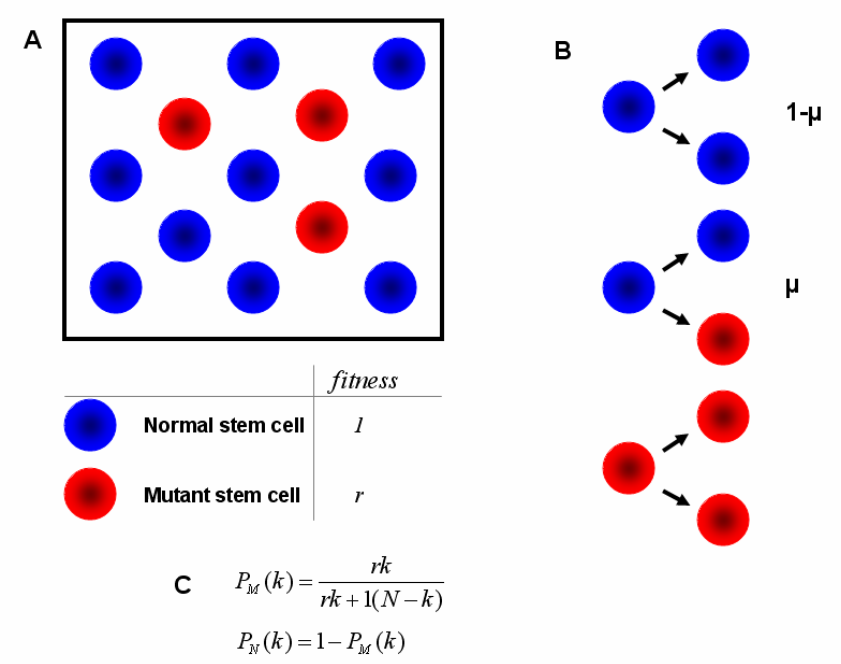

Figure 2. Schematic representation of hematopoietic stem cell dynamics. The number of active stem cells is considered to be constant (A) and there are both normal (blue) and mutated (red) cells. (B) When a normal HSC divides, with probability 1-μ, it gives rise to two normal HSC, but with probability μ, one of the daughter cells is mutated. Mutated HSC only give rise to more mutated cells. (C) Cells are chosen for reproduction proportional to fitness; whenever there are k mutated cells with relative fitness r in a population of size N, the probability that one is chosen is given by $P_M(k)$ whereas the probability that a normal cell is chosen is given by $1 - P_M(k)$.

Since normal HSC replicate approximately once per year [19,27], in one year approximately ~400 HSC divisions take place. This provides a natural time scale for our evolutionary dynamics. By repeating the Moran process many times, we can compute

probability distribution functions that illustrate the evolutionary histories of mutations in a population of virtual individuals [37].

Given the very small mutation probability per cell division of 10^{-7}, and once a mutated cell appears in the HSC population, the Moran process has two absorbing states (of course, if we wait an arbitrarily long time only one absorbing state exists in the presence of mutations): either the clone expands invading the entire population or it becomes extinct. However, during the finite lifetime of each individual organism, clonal invasion is rarely achieved, such that intermediate stages are often observed. Moreover, it is often the case that the appearance of disease does not require that all the HSC are mutated (see below). A small population of mutated HSC (e.g. cancer stem cells, CSC) may be enough to induce disease, if not a lethal burden [37,38]. The model predicts that a mutant HSC clone can invade the whole population, go extinct or persist at relatively 'stable' levels that may or may not cause disease (Figure 3).

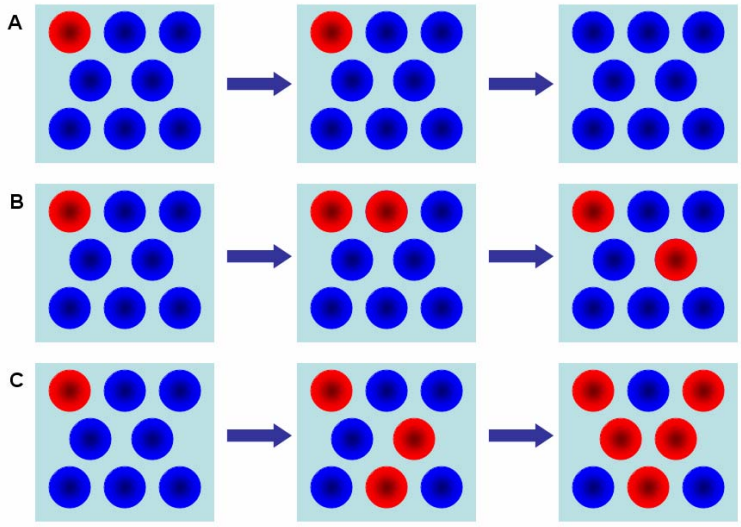

Figure 3. Outcome of stochastic stem cell dynamics. The Moran process encompasses 3 possible outcomes in a finite lifetime. The mutant cell population may go extinct (A), remain latent (B), or invade the population leading to disease (C).

In silico studies of such a population of cells show that starting from one mutated HSC, the probability of invasion increases with higher fitness, while the chance of extinction falls down. However, even for a fitness advantage of 2 - considered by many to be a high fitness advantage [39,40] - such a cell has a 50% probability of going extinct. Thus, the

fitness conferred by the mutation is clearly an important parameter when describing the phenotype of cancer cells. Unfortunately, it has been easier to define "*r*" than to provide experimental estimates of its value.

For various hematopoietic tumors, a fraction of the cells in the bone marrow have to be abnormal for the tumor to be clinically detectable or defined. For example, at least 10% plasma cells have to be present for myeloma to be diagnosed and 20% bone marrow blasts are necessary for a diagnosis of acute leukemia [38]. If we take these thresholds into consideration, it can be shown that once the mutant clone burden has increased to reach, say 20% of N_{SC}, stochastic clonal extinction, although rare, is still possible [37]. The fitness advantage of mutant cells also plays a crucial role in determining the time necessary for invasion or to reach the threshold for diagnosis. The evolutionary histories of these cells are described by probability distribution functions that are always 'one humped' functions of time [37,38]. The variance of the distributions depends on the fitness associated with the mutation. For a small fitness advantage, the distributions are very wide but they become narrower with increasing fitness advantage. Our results suggest that for a mutation with a low fitness advantage, the 'average' time to reach a diagnostic threshold or invasion (the mean of the corresponding distribution) is of the order of the variance of the distribution, as such lacking a precise meaning. Of course, these observations relate to disorders where a single mutation may be enough to explain some aspect of the disease (e.g. CML in chronic phase, [41,42,43]). However, a corollary of this model is that for a single gene mutation, knowledge of the time to development of the disease can give an estimate of the fitness advantage associated with that mutation. For example, if retinoblastoma were due to mutations in the *Rb* gene only, the fitness advantage of the mutation must be >1.7 to be compatible with the time frame in which the disease appears [37].

Although the concepts of tumor progression and clonal expansion are well established features in cancer, perhaps the idea of stochastic extinction of a clone or latency are less obvious and one may think that these are artificial results of the model. However, there is clinical evidence for 'spontaneous' elimination of malignant clones. In our view, one of the most striking cases is that of transient leukemia (TL) that often develops in children born with Down's syndrome [44,45]. Some studies suggest that

perhaps up to 10% of children with Down's syndrome develop TL, usually within 5 days of birth [46]. This potentially lethal disease clearly affects an early progenitor cell in hematopoiesis (if not the HSC) [44,46]. However, in up to 85% of cases, the disease resolves with minimal or only supportive therapy [45].. Our model of stochastic dynamics can explain this behavior. In addition, there are reports of 'spontaneous' resolution of myelodysplastic syndromes in patients [47]. Finally, the model suggests that malignant clones can experience latency and stability whereby they do not change in size appreciably. The best evidence for this is provided by a cohort of patients with essential thrombocythemia. In this group, the size of the $JAK2^{V617F}$ clone was determined serially and shown to be stable over several years [48], also compatible with our model of stochastic HSC dynamics.

Symmetry of stem cell replication and fitness

A *sine qua non* property of stem cell replication is self renewal. The fate of the daughter cells defines the symmetry of division: 'symmetric' division gives rise to two cells that have the same fate (they both either differentiate or retain stem cell properties), but when the daughter cells have different fates, the division is considered 'asymmetric' (Figure 4A) [9].

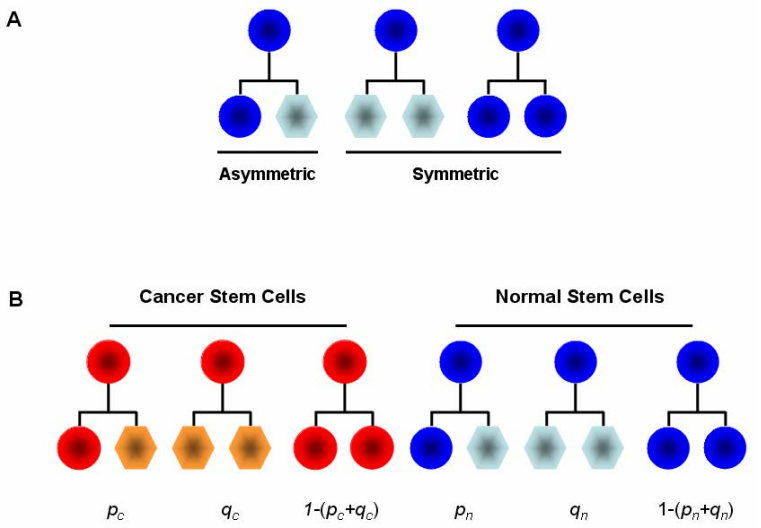

Figure 4. Hematopoietic stem cell reproduction. HSC can divide symmetrically or asymmetrically (A) depending on the fate of the daughter cells. Mutant HSC (CSC) may have different probabilities for self-renewal compared to normal HSC. A higher probability of self-renewal in the mutant cells appears as a fitness advantage, as mutant cells preferentially outgrow normal cells.

In principle, asymmetric division should be enough to maintain tissues by simultaneously keeping the HSC population constant and feeding downstream compartments. However, this would not allow HSC expansion that is necessary during ontogeny or in response to injury or bone marrow transplantation. There is experimental evidence from DNA methylation patterns that all 3 forms of SC division depicted in Figure 4A can occur depending on the demands imposed on the SC pool [11,49].

The determinants of the (a)symmetry of HSC replication are not completely understood, although the interactions with the stem cell niche and signals via the Notch, Hedgehog and Wnt pathways (including β-catenin) seem to be quite important [50,51,52]. Recent work in model organisms is starting to decipher the potential impact of mutations on the (a)symmetry of stem cell replication. Various genes appear to be important in this respect, including *partner of inscuteable (PINS)* [53], *lethal giant larvae (LGL)*[54], *HUGL-1*and *adenomatous polyposis coli (APC)*. Mutations in *PINS, LGL and HUGL-1* result in the formation of tumor like tissue in these animals.

In order to evaluate the impact of mutations that alter the symmetry of HSC replication, we have developed the model illustrated in Figure 4 [55]. The process of HSC replication is depicted by parameters p and q that define the probability of (a)symmetric HSC replication. A normal HSC divides asymmetrically with probability p_n while, with probability q_n, the cell divides symmetrically to give rise to 2 differentiated cells. Therefore, with probability $1-p_n-q_n$, the cell divides symmetrically to give rise to two HSC (self renewal). Mutations confer to the cell fitness r and cells are chosen for reproduction according to their fitness (Figure 2C). The dynamics resembles a Moran process in that the total cell population is kept constant, but cells may be forced to divide symmetrically to replenish the pool if a cell is lost [55]. What would be the impact of a mutation that increases the probability of self-renewal to the mutated cell compared to that of a normal HSC (i.e. $(p_c+q_c)<(p_n+q_n)$)? It can be shown that these types of mutated cells will take over the compartment, even in the absence of a selective advantage ($r=1$). Such mutations by themselves enable the mutant to invade the population and impart an effective 'reproductive fitness' advantage to the cells. Naturally, if the mutation also gives a selective advantage whereby the mutant cells are chosen more often for reproduction, the average time needed for fixation will be

significantly reduced [55]. Therefore, mutations that increase the self-renewal capability of cells may be an early event in cancer. Indeed, these mutations favor the mutated population to outgrow in number the normal cell population, thereby increasing the population of cells at risk, with the possibility to accumulate additional mutations and progression.

Because of their location at the root of the hematopoietic tree, HSC would be expected to be the only ones exhibiting long-term self renewal capability, as progenitor cells can self-renew but for a limited time [10]. However, it has been shown that acute myeloid leukemia can arise in progenitor cells such as CFU-GEMM that re-acquire stem cell-like properties and can drive the tumor [56,57]. Our modeling suggests that such a mutant (progenitor) cell must acquire the potential for long-term self-renewal at an early step in its path to cancer, otherwise it will be eliminated relatively rapidly down the hematopoietic cascade [33,58].

Stem cell pool, longevity and cancer

Similar to normal blood cells, hematopoietic tumors are also driven by cancer stem cells (CSC) [59]. CSC can arise from HSC by mutations, although not all CSC necessarily result from the malignant transformation of normal stem cells. Hence, HSC are dangerous cells, as mutations in them lead to cancer, giving rise to the myeloproliferative disorders (e.g. CML and polycythemia vera) [3,29]. Normally, cells are at highest risk of acquiring mutations during DNA replication. The rate of replication of the HSC *decreases* with mass as $B_c = B_0 M^{-1/4}$ [19]. On the other hand, the size of the active stem cell pool *increases* with mass ($N_{SC} \sim M^{3/4}$). For the disease to be detectable mutations that transform HSC into CSC must occur and subsequently the clone has to expand during the lifespan of the mammal. Interestingly, the expected mammalian lifespan (L_E) also scales allometrically (*increases*) with mass ($L_E = L_0 M^{1/4}, L_0 = 8.6$) [60,61]. Finally, the probability of reaching a given fraction of mutated cells should *decrease* as the size of the active stem cell pool expands. In order to explore the impact of these potentially conflicting variables on the development of CSC, we performed simulations of HSC dynamics taking these variables into consideration. For the purpose of these simulations, any species is characterized by the average adult mass characteristic

of that species and we estimated N_{SC}, L_E and B_c using the above definitions. The size of the species-specific active stem cell pool was kept constant during the lifetime of a given mammal. We considered that the mutation rate, ($10^{-7} \le \mu \le 10^{-6}$) is constant across all mammals [36,62] and back mutations do not occur. A contamination threshold of 20% was assumed to be necessary to define disease in all species.

In Figure 5, we show results of such simulations as a function of both mass and fitness advantage of the mutant cell. The data suggest that larger mammals are better able to resist clonal expansion associated with mutations that are either neutral or marginally advantageous.

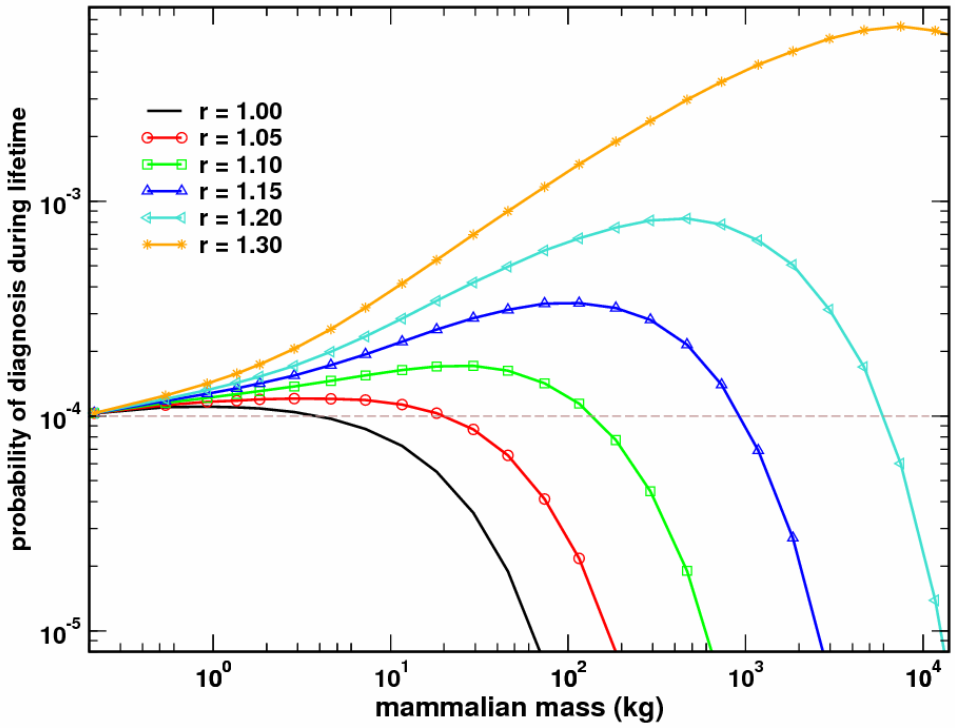

Figure 5. Malignant hematopoietic stem cell disorders across mammals. The probability of a neoplastic HSC disorder is plotted as a function of mass and for different values of the relative fitness of mutated cells. Each species is defined by the average mass of an adult in that species while the expected lifetime is also a function of mass. In general, the probability of diagnosing malignant HSC disorders increases with mass and no mammal is more protected from these disorders than the smallest rodents.

However, for mutations with a significant fitness advantage, larger species are worse off compared to smaller mammals. This is so because, once such a mutation occurs, the probability of diagnosis does not necessarily decrease for larger mammals [61]. On the other hand, for any species, the expected number of mutations (n_μ) within the HSC pool (i.e. the population at risk), is given by: $n_\mu = \mu N_{SC} B_c L_E \approx n_0 M^{3/4}$ which favors smaller mammals [61]. As a result, the occurrence of the first mutation becomes the decisive factor. Larger mammals, with their larger active stem cell pool are protected from the expansion of *neutral* mutations but not from advantageous mutations. Our results are robust with respect to the mutation rate and the fraction of mutated cells necessary to cause disease within the HSC pool [61].

These *in silico* results are supported by clinical observations both in humans and in the veterinary setting. A search of the mouse tumor database (http://www.nih.gov/science/models/mouse/resources/mtbdb.html) suggests that spontaneous development of a myeloproliferative disorder has not been reported in this mammal. The incidence of CMPD is uncommon in dogs that share our environment and presumably are exposed to the same level of background radiation as humans [63]. Finally, CMPD are characteristically uncommon in humans younger than 20 years of age (<10% of cases of CML occur in people younger than 20 years of age), again compatible with our model [38]. An unavoidable conclusion of our modeling is that the human species is a victim of its own success: while the average human lifespan provides the largest deviation from the predicted allometric lifespan (being much longer than predicted), this comes at the price of higher risk of CMPD. Our modeling also naturally explains why the incidence of cancer tends to increase with longevity.

In conclusion, a complete understanding of HSC behavior must include the dynamic behavior of these cells. Conceptually, HSC behavior can be rationalized using allometric principles together with stochastic dynamics. The small size of the active HSC pool calls for a probabilistic description of these cells which can have a major impact on the dynamics of this cellular compartment. Dynamic considerations also help us understand clinically relevant observations pertaining to various neoplastic and acquired genetic disorders of hematopoietic stem cells. In principle, these considerations should apply to other (non-hematopoietic) stem cell compartments as well.

Acknowledgements

Financial support from Mayo Foundation (DD) and FCT-Portugal (JMP) is gratefully acknowledged.

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