Chapter 11

Evolutionary Dynamics of Mutations in Hematopoietic Stem Cells and Beyond

David Dingli, Arne Traulsen, and Jorge M. Pacheco

Abstract Mutations are a natural consequence of the interactions of our genome with genotoxic agents and imperfections in the DNA replication machinery. Every cell is at risk of mutations and therefore the probability of acquiring mutations is increasing with population size. However, the impact of a mutation depends on the type of cell where it occurs and the average lifetime of that cell. Tissue architecture is organized in such a way that many mutations will have no consequence, although the cell harboring them may expand into a detectable clone. We will use the known architecture and dynamics of hematopoiesis to describe the evolution of mutant clones in age structured populations and show why the appearance of well recognized mutations is inevitable even if usually of no consequence. Most mutant populations merely cause transient ripples in a tissue. However, whenever mutations occur in stem cells or other primitive cells, the associated clones can have long lasting consequences and may lead to disease.

Keywords Hematopoiesis · Mutations · Retroviruses · Neutrophils · Apoptosis · CFU

D. Dingli (✉)
Division of Hematology and Department of Molecular Medicine, Mayo Clinic, Rochester, MN 55905, USA
e-mail: Dingli.david@mayo.edu

Introduction: Multicellularity and Mutations

The cellular genome is under continuous attack. Environmental genotoxic agents abound and include radiation from various sources (background, cosmic, medical use, and radioactive fallout); chemicals including therapeutic agents (e.g. alkylating agents and benzene), and viruses (e.g. integrating retroviruses). Moreover, although DNA polymerases are highly accurate, they exhibit an error rate of around 1×10^{-9}/base/replication or $\sim 1 \times 10^{-7}$/gene/replication (Araten et al., 1999). The human haploid genome is circa 3×10^9 base pairs in length, and in our body, there are approximately 10^{14} cells. Therefore, a simple calculation shows that we are riddled with mutations: indeed, there is probably at least one cell harboring *any one* possible mutation in our body (Traulsen et al., 2010). However, as in real estate, location is everything and the impact of a mutation depends on the DNA sequence context as well as on the type of cell: many mutations have no consequence on the phenotype of the cell and a mutated gene can be neutral in one cell but may alter the reproductive fitness of differentiated, daughter cells downstream, exhibiting a different phenotype (Dingli et al., 2009; Lenaerts et al., 2010).

Acquired (somatic) mutations may lead to the development of clones of cells that are at the root of neoplastic transformation (Hanahan and Weinberg, 2011). Serial accumulation of mutations can produce a cell that ignores the mechanisms which regulate growth control and in addition may acquire an ability to invade other tissues – i.e. the full cancer phenotype (Hanahan and Weinberg, 2011).

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Population Structure and Dynamics of Hematopoiesis

It should be evident from the prior discussion, that mutations are inevitable. Therefore, one would expect that evolution has selected for a tissue architecture that minimizes the risk of retention of mutant populations. The probability that a particular mutation occurs in a given tissue is proportional to the population of cells at risk, the mutation rate and the average lifetime of cells in that population. Most tissues (including hematopoiesis and epithelia) have a tree like architecture in which the vast majority of cells have a relatively short lifetime. At the root of this tree lie (tissue specific) stem cells that are operationally defined by their ability to self-renew and give rise to progeny cells that can differentiate and repopulate the entire variety of lineages, and are hence able to generate and maintain a specific tissue. In general, the stem cell population is but a tiny fraction of the cells making every tissue, but the definition of a stem cell does not require this characteristic. Stem cells generally divide slowly (compared to other cell lineages of the same tissue) but remain in the body, comparatively, for a very long time. Their division gives rise to more differentiated cells that often replicate at faster rates but which contribute to tissue maintenance for shorter periods of time. Mature cells in the tissue are eliminated at a constant rate by, e.g., apoptosis in hematopoiesis or by shedding from the surface of epithelia. Given that hematopoiesis is perhaps the best understood tissue with respect to its architecture and dynamics, we will use it to highlight the dynamics of mutations in such tree-like structured cell populations.

Hematopoiesis Has Many Cells and Many Cell Types

Under physiologic conditions, our bodies replace approximately 1% of circulating red blood cells every day. Neutrophils have a lifetime of several hours whereas platelets survive for around 10 days in the circulation. Therefore, hematopoiesis constitutes a huge and fascinating undertaking, with the production of $\sim 3 \times 10^{11}$ cells per day. In line with our previous discussion, this process 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 differentiate along the various lineages that result in all types of blood cells (McCulloch and Till, 1964). Both experimental as well as theoretical analyses suggest that only a fraction of the HSC pool is actively contributing to hematopoiesis: in humans estimates suggest that this pool consists of around 400 cells (Dingli and Pacheco, 2006). HSC divide slowly, on average once per year (Dingli and Pacheco, 2006). Linking the HSC and mature blood cells is a hierarchically organized process where cells divide and become increasingly differentiated (Fig. 11.1).

One can capture the dynamics and architecture of hematopoiesis by considering two fundamental processes: cellular reproduction and differentiation (Fig. 11.1). In a recently proposed mathematical formulation of this process (Dingli et al., 2007a), we considered that cell renewal (reproduction) and differentiation are stochastic and coupled processes. Using such a model to capture the dynamics of hematopoiesis makes it possible (i) to infer the cell of origin of any mutant clone when we know its size and (ii) estimate the average lifetime of such a clone for a mutation that does not alter the reproductive fitness of cells (neutral mutation) (Dingli et al., 2008a; Dingli et al., 2008b; Traulsen et al., 2007).

This average lifetime will generally be a lower limit: a clone with a mutation that provides a fitness advantage will be expected to survive for longer (Dingli et al., 2008a; Traulsen et al., 2007).

One can speculate as to why this architecture has been selected for by evolution since it is now ubiquitous in most tissues of multicellular organisms including epithelia (e.g. colonic crypt) (Johnston et al., 2007). The current view is that such an organization of cells reduces the probability that mutant clones will invade the tissue cell populations, and therefore serves as a deterrent against cancer. One can see how the relatively short lifetime of most cells in hematopoiesis will probably negate them the possibility of acquiring all the mutations necessary to develop the full cancer phenotype. As a result, although mutant clones continuously emerge, they may or may not proceed all the way to the development of cancer, e.g., a myeloid neoplasm (Dingli et al., 2008c; Lenaerts et al., 2010). This probability depends in part on whether mutated cells exhibit any (fitness) advantage compared to normal cells. In situations where mutation(s) increase the probability of self renewal of progenitor cells, then the resulting

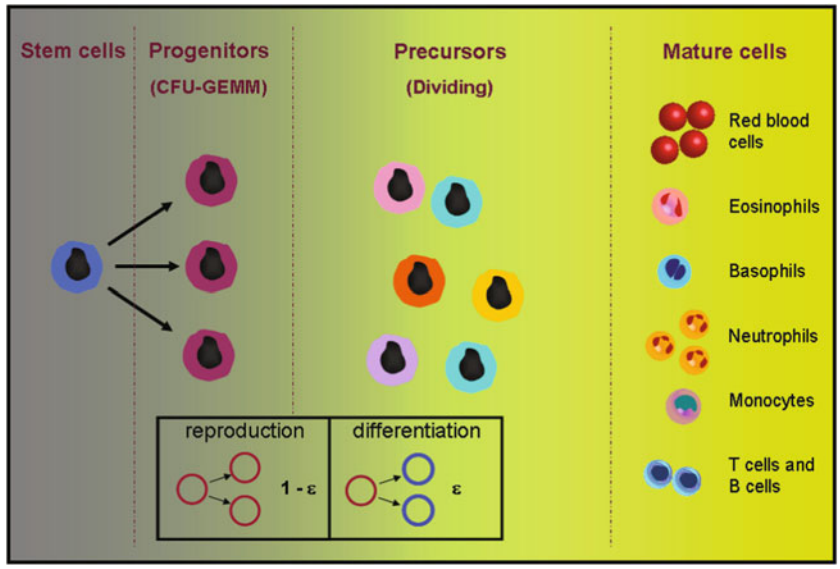

Fig. 11.1 The tree-like structure of hematopoiesis. Hematopoiesis exhibits a hierarchical architecture so characteristic of general body tissues. At the root of this tree-like structure one finds the tissue specific stem-cells, which can self-renew (reproduction) and differentiate (see *top box*) into all other types of hematopoietic cells. The figure illustrates specifically the most common hematopoietic cell types, from progenitors to mature cells. Along this path of differentiation, we also find the so-called precursor cells. In the mathematical model of hematopoiesis referred to in the main text, cell reproduction and differentiation constitutes a coupled stochastic event, occurring with probability 1-ε and ε, respectively, as illustrated

fitness advantage may be enough to enable the clone to expand, leading to disease (Dingli et al., 2008a). However, the stochastic nature of hematopoiesis also means that clonal extinction is possible even when mutant cells have a fitness advantage compared to normal cells, though in this case the probability of clonal extinction decreases as the fitness of the mutant clone increases (Dingli et al., 2007b). Below we shall make use of this model to illustrate why small clones with well known genetic defects are in essence an inevitable consequence of population size and the known mutation rate. In addition, we will show how these clones can come and go, to be replaced by new ones – observations that also have clinical significance.

A Normal Mutation Rate Still Equates with Many Mutations

While 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 CFU-GM is significantly larger. There are approximately 1.0×10^5 CFU-GEMM cells and even more CFU-GM cells ($\sim 10^8$). These cells form a substantial component of the peripheral blood progenitor cells infused in patients for autologous stem cell transplantation and are responsible for the rapid recovery of hematopoiesis after potentially myeloablative chemotherapy. Our modeling suggests that CFU-GEMM, on average, contribute to hematopoiesis for ~60 days (range 40–340 days) and on average they replicate once every 50 days (range 35–285 days). The replication rate of CFU-GM, in turn, is significantly faster (Dingli et al., 2007a). When dealing with a large cell population where cells exhibit substantial replication rates, it should be no surprise that many cells with specific defects will appear. However, we must not forget that (i) the total cellular output from each of these cells tends to be small, and (ii) the average lifetime of these cells decreases rapidly as they continue their path of differentiation. Although mutations can occur in any cell that is dividing, the majority of mutations will occur in the larger populations of cells that occupy positions in later stages of the hematopoietic hierarchy and as a result they will have very little clinical significance.

Such clones will generally be small in size (often below current detection limits) and short lived since they will be quickly washed out of hematopoiesis. Therefore, one can quickly determine that what matters are mutations within the HSC and the early progenitor cells up to the CFU-GEMM since mutations in these can potentially lead to disease. Mutations in more mature cells will lead to inconsequential ripples in hematopoiesis, unless the mutation drastically alters the self-renewal properties of the cell and enable it to grow rapidly.

Mutation Dynamics in Hematopoietic Stem and Progenitor Cells

Mutations that arise within hematopoietic stem cells are associated with well defined neoplastic and non-malignant diseases including chronic myeloid leukemia (CML) (Goldman and Melo, 2003), chronic myeloid neoplasms (CMN) such as polycythemia vera (PV) (Vainchenker and Constantinescu, 2005) and the enigmatic disease paroxysmal nocturnal hemoglobinuria (PNH) (Luzzatto et al., 1997). However, mutations in more committed progenitor cells (e.g. CFU-GM) can lead to the re-acquisition of enhanced self-renewal and stem cell like properties by these cells and as a consequence potentially lethal disease such as acute promyelocytic leukemia (APL) (Guibal et al., 2009). Mutations such as *MLL-AF9* and *MOZ-TIF2* can also enhance the self-renewal of more committed progenitor cells and lead to acute myeloid leukemia.

In the following, we will illustrate the dynamics of mutations in hematopoiesis using three extensively investigated somatic mutations that are specifically associated with potentially life-threatening blood disorders: (i) the inactivating mutations in the *PIG-A* gene that are characteristic of paroxysmal nocturnal hemoglobinuria (PNH) (Araten et al., 1999), (ii) the gain of function mutation V617F of the *JAK2* gene characteristic of myeloproliferative neoplasms (MPN) (Vainchenker and Constantinescu, 2005) and (iii) the Philadelphia chromosome, a translocation that results in the *BCR-ABL* fusion gene characteristic of chronic myeloid leukemia (CML) (Bose et al., 1998). All of these mutations have been observed in the absence of disease.

Cells with the PNH Phenotype

Virtually every healthy person has a small population of neutrophils and erythrocytes that lack glycosylphosphatidyl inositol linked (GPI) proteins on their surface (e.g. CD55 and CD59) (Araten et al., 1999). This phenotypic feature is due to acquired somatic mutations in the *PIG-A* gene that is critical for synthesis of the GPI anchor that targets and retains these proteins on the cell surface (Luzzatto et al., 1997). In contrast to *JAK2V617F* and *BCR-ABL* (see below), which are gain of function mutations, mutations in *PIG-A* lead to a reduction or complete loss of GPI synthesis. Therefore their spectrum is quite heterogeneous (Araten et al., 1999) with every patient having a rather 'personal' mutation, since it is much easier to break something delicate than to make a superior product!

The appearance of mutated GPI-red cells and neutrophils can occur via two mechanisms: (i) a *single* founder mutation in a common progenitor (such as a CFU-GEMM), or (ii) two *independent* but almost simultaneous mutations in cells committed to either neutrophil or erythrocyte differentiation (Dingli et al., 2008a; Traulsen et al., 2007). The rate of accumulation of mutations in *PIG-A* is $\sim 5 \times 10^{-7}$ per replicating cell (Araten et al., 1999). Therefore, the probability that in any interval of time there is a CFU-GEMM cell with a mutation in *PIG-A* is given by the product of the average number of CFU-GEMM cells, their replications in the time interval and the mutation rate for *PIG-A*. Plugging in the numbers leads to a probability of almost unity (Araten et al., 1999). Any CFU-GEMM cell contributes to hematopoiesis for a finite period of time and therefore a *PIG-A* mutant cell will expand into a clone but will be 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: in essence a ripple in hematopoiesis. Mutations in *PIG-A* would arise much less frequently in the HSC: however in such situations, the mutant clone can expand and lead to disease (PNH), either as a result of a fitness advantage imposed by the local microenvironment (Luzzatto et al., 1997) or even by neutral drift (Dingli et al., 2008b). Indeed, we have previously shown that, even without selection, the population frequency of PNH in the United States is not far from what our model would predict (Dingli et al., 2008b).

JAK2*V617F* in Health and Disease

Many patients with a chronic myeloproliferative neoplasm have a gain of function mutation in *JAK2* (*JAK2V617F*) (Vainchenker and Constantinescu, 2005). It has been shown in animal models that the *JAK2*V617F mutation in a HSC gives a phenotype similar to polycythemia vera (PV), sometimes with bone marrow fibrosis (Jamieson et al., 2006). The coding region of the *JAK2* gene is 5285 base pairs in length and a unique base substitution at position 617 converts a codon for valine (V) to a codon for phenylalanine (F). Only a guanosine to uracil substitution G→U in the first position of the codon can effect this amino acid substitution. The normal mutation rate is 1×10^{-9}/base/replication, and the probability that U is replaced by G is 1/3. Therefore the overall probability that this specific mutation occurs is $\sim 3 \times 10^{-10}$ per replication. If we consider that the average lifetime of a human is 70 years, then the probability that this specific mutation occurs at least *once* within the HSC pool in a typical human lifetime is given by $400 \times 70 \times 3 \times 10^{-10}$. This probability translates into a lifetime incidence of approximately 1:100,000 which is very close to the actual incidence of polycythemia vera in the general population. The *JAK2V617F* mutation allows the cell to become independent of growth factor stimulation and therefore confers a fitness advantage onto the mutated HSC – enabling it to expand gradually into a large clone. In general, the rate limiting step is the appearance of the *first* mutant cell, and therefore it is not surprising that our estimate above is quite close to the prevalence of clinical disease – what matters is the appearance of the first mutant HSC. From then on, disease evolution is quite straightforward.

Recent studies show that the *JAK2V617F* mutation is quite common in the general population – it may be found in up to 0.94% of patients hospitalized for a non-hematologic disorder (Xu et al., 2007) and may be present in 0.2 % of an un-selected population of healthy individuals (Nielsen et al., 2011). Similar findings were reported in blood donors with a high hematocrit. The vast majority of these individuals have normal blood counts and a very low allele burden (<5%). Clearly, most of these individuals do not have an overt disease such as an MPN. An important question then is how to explain the presence of a pathogenic mutation in people who do not have the associated disease. One possibility is that some of these individuals are in a prodromal stage of MPN: however, this cannot be the most likely explanation; otherwise the prevalence of MPN should be much higher than what is observed. Another possibility is that the mutation has taken place not in a HSC, but in a CFU-GEMM cell (just as we have earlier suggested 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 but eventually disappear without leading to disease. Unfortunately, at present the time course of these *JAK2V617F* clones in subjects without MPN is as yet unknown. However, it is reasonable to expect that the mutation will also provide a reproductive fitness advantage to the CFU-GEMM cell including enhanced self-renewal of the cells. Therefore this mutation has the ability to increase and prolong the contribution of a mutant CFU-GEMM to hematopoiesis. So, one can speculate that if the *JAK2V617F* occurs in an HSC, the growth advantage is so great that the resulting clone becomes pre-dominant, and often takes over hematopoiesis leading to disease while the same mutation in a CFU-GM will give rise to a transient clone (a small ripple). When the mutation occurs in a CFU-GEMM, the resultant clone size may be significant and persist for a considerable time (Fig. 11.2).

Cells with the Philadelphia Chromosome

Chromosomal translocations that lead to the generation of fusion genes are among the most powerful oncogenic events. Chromosomal abnormalities are found with a frequency of around 1.0×10^{-5} per cell per replication (Lucas et al., 1997). However, the incidence of specific chromosomal translocations is much lower. Chronic myeloid leukemia (CML) is characterized by the Philadelphia chromosome (t(9;22)(q34;q11))(Rowley, 1973) that produces the *BCR-ABL* fusion gene. It is possible to predict the probability that such a translocation occurs in a HSC in the average lifetime of a human (~70 years). The average cumulative probability of having a HSC with the Philadelphia chromosome (P_{Ph}) is given by: $P_{\mathrm{Ph}} = N_{\mathrm{SC}} \cdot R \cdot \mu_{\mathrm{T}} \cdot \mu_9 \cdot \mu_{22}$ (Traulsen et al., 2010). Here N_{SC} ~400 refers to the number of active HSC (Dingli and

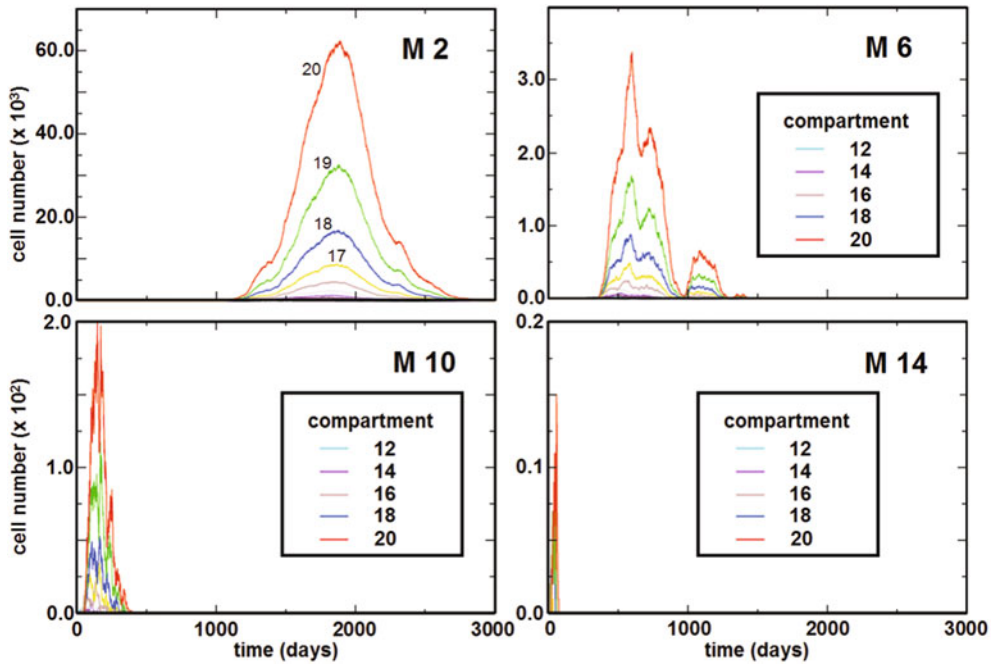

Fig. 11.2 Clonal dynamics of mutated cells. The figure provides direct information of clone size (given by the cell number count) in a given compartment (indicated by *lines* with different *colors*), originating from an initial mutation occurring in compartments 2 (M2, *top left*), 6 (M6, *top right*), 10 (M10, *bottom left*) and 14 (M14, *bottom right*). In all cases, the mutated cells have a fitness advantage compared to normal cells. As displayed in the different panels, mutations occurring in more primitive compartments give rise to larger clones that survive for longer, but also taking a longer time to appear in the circulation. Smaller clones, instead, are caused by mutations in more differentiated (precursor) cells, appear more rapidly in the circulation, but reach a smaller size while surviving also for a shorter period of time, and simply provoke small ripples in an ocean of cells (note that the scale on the *y*-axis is different in the four panels)

Pacheco, 2006), $R = 70$ is the average number of cell divisions of each HSC during the lifespan of the organism(Dingli and Pacheco, 2006), $\mu_T = 10^{-5}$ is the probability of any chromosomal translocation per cell per replication, $\mu_9 = 0.0011$ is the conditional probability that chromosome 9 breaks at band q3 and $\mu_{22} = 0.0056$ is the conditional probability that chromosome 22 breaks at band q1. The reciprocal of P_{Ph} gives roughly the incidence of the translocation in the population, namely 1.7:100,000 individuals. This result is very close to the reported incidence of the disease in the United States (1.5 per 100,000 per year, SEER database). Here, we are assuming that one or a few leukemic stem cells are enough to drive the chronic phase of CML (Dingli et al., 2008c; Dingli et al., 2010; Lenaerts et al., 2010; Pacheco et al., 2009), consistent with experimental observations that *BCR-ABL* expression in a *single* HSC can result in a disease similar to chronic phase CML in animal models (Daley et al., 1990).

Using the same arguments discussed previously for *PIG-A* and *JAK2V617F*, the Ph' chromosome can also occur in CFU-GEMM cells. Given that an average human adult has $\sim 10^5$ CFU-GEMM, that on average replicate approximately 7 times per year, one can determine a 50-fold increase in incidence of this abnormality compared to the incidence of the same mutation in the HSC pool, that is, 1:2300 *healthy* adults will have a CFU-GEMM cell with the Ph' chromosome. Naturally, the probability that the translocation will occur is higher if we consider cells downstream of the CFU-GEMM pool. However, we expect such a clone to expand transiently and then disappear and perhaps providing a simple explanation for the observation that healthy people can have detectable Ph' positive clones but no disease (Bose et al., 1998).

Distinct Somatic Mutations May Co-exist Within Hematopoiesis

There are reports of patients with myeloproliferative neoplasms who may harbor two distinct clones, i.e., one with *JAK2V617F* and another with a different genetic lesion such as trisomy 8 or 9 (Beer et al., 2009). Patients with PNH may also have distinct populations of blood cells with two different mutations in *PIG-A* (for instance when they have PNH-II and PNH-III cells) (Bessler et al., 1994a, b). The null hypothesis is that this may happen simply by chance, with each mutation being in an independent clone: for neutral mutations, this is the more likely scenario (see Fig. 11.3a). However, if the first mutation confers to the clone a large fitness advantage (Fig. 11.3b), the chance of a second mutation will depend on two factors: (i) the mutation rate may have changed due to the presence of the first mutation itself (e.g. increased genomic instability in cells expressing *BCR-ABL*) and (ii) the size of the mutant clone, which will be gradually increasing. One can show mathematically that it is much more likely that a new clone with a second independent mutation (i.e. same gene or a different gene) occurs in a cell *without* the first mutation unless the first mutant population grows to a very large size (Dingli et al., 2008a; Traulsen et al., 2007). In other words, scenario **A** is much more likely than scenario **B** (Fig. 11.3).

Under these circumstances, the clones may co-exist, especially if their fitness is comparable (Dingli et al., 2007b). One specific example of this situation also comes from PNH. Two patients have been described who had a mutation in *HMGA2*, which almost certainly provides a growth advantage to the cells (Inoue et al., 2006). Similarly, a few patients with both *BCR-ABL* and *JAK2V617F* have been reported: in such cases it

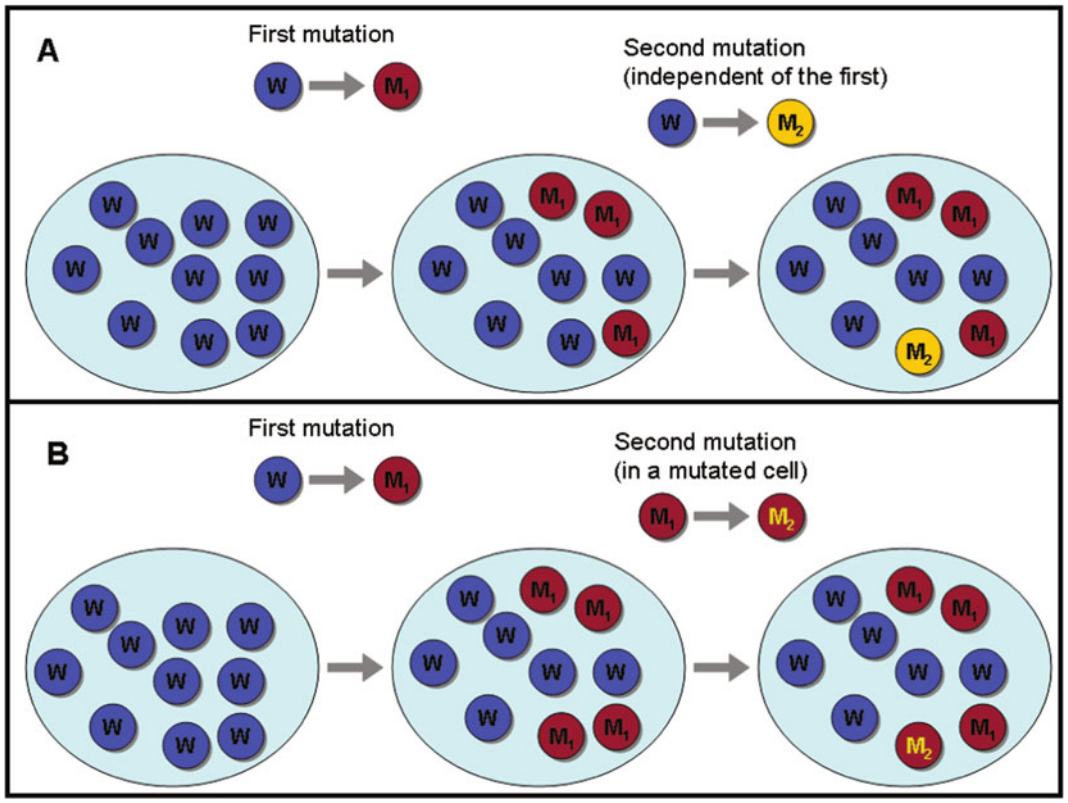

Fig. 11.3 Multiple mutations in cell populations. The probability that mutated cells acquire additional mutations depends on the number of cells at risk, which in turns depends on the fitness of the original mutant clone. The mathematical model allows us to infer that, whenever we find two different mutations, the scenario depicted in the *top panel* (**A**) is the most likely, that is, the mutations happen independently and act on the wild type cell population, instead of occurring sequentially on the same cell, as illustrated in the *bottom panel* (**B**)

is reasonable to assume that the double mutant clone will have an extra selective advantage. Once again, the probability of a specific mutation leading to a single amino acid substitution, say in a receptor tyrosine kinase, is very small – indeed much smaller than the probability of loss or gain of gross chromosome material (such as del(20q)). This could explain, for instance, why Beer et al. (2009) could not identify any patient with a clone that had simultaneous mutations in two different tyrosine kinases (e.g. *JAK2*V617F and *MPLW515L*).

Final Remarks

Structure begets function but also implies dynamics. These three characteristics are intimately intertwined, but while structure and function have been well studied, the dynamics has been often neglected. However, tissues are changing structures and understanding the dynamics is of fundamental importance. Here we have used hematopoiesis as a model to illustrate how mutations that are associated with specific diseases will inevitably arise in tissues. However, the presence of mutation(s) does not equate with disease, although the term 'somatic mutations' is often used synonymously with cancer. Many mutations will be inconsequential since they occur in the wrong cell or the cell will not survive long enough to expand and cause disease. In this respect, the architecture of tissues naturally acts to reduce the incidence of cancer – the short lifetime of most cells effectively reduces the risk of the serial accumulation of mutations in the same cell. This architecture functionally reduces the fitness of cells as they differentiate further (Dingli et al., 2008a). The fact that multiple mutations are required for the cell to achieve the full cancer phenotype serves as an additional deterrent against the development of cancer.

A mathematical analysis of the hierarchical structure of hematopoiesis and other tissues is essential for understanding the incidence and dynamics of mutant clones and may help generate hypotheses on promising interventions. The appearance of mutations in large cell populations is inevitable, but most of them will simply have no consequence. They simply create small ripples in an ocean of cells. Our ability to identify *small* mutant populations in humans that may or may not lead to disease will continue to increase. We must remain vigilant and avoid diagnosing such individuals with disease and they are treated for a disease that does not exist or worse still develop complications from a therapy that was not even needed. Our analysis of the evolutionary dynamics of mutations in structured populations should apply to most tissues in humans since most tissues have a similar hierarchical organization.

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