Can a minimal replicating construct be identified as the embodiment of cancer?

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Abstract

Genomic instability is a hallmark of cancer. Cancer cells that exhibit abnormal chromosomes are characteristic of most advanced tumours, despite the potential threat represented by accumulated genetic damage. Carcinogenesis involves a loss of key components of the genetic and signalling molecular networks; hence some authors have suggested that this is part of a trend of cancer cells to behave as simple, minimal replicators. In this study, we explore this conjecture and suggest that, in the case of cancer, genomic instability has an upper limit that is associated with a minimal cancer cell network. Such a network would include (for a given microenvironment) the basic molecular components that allow cells to replicate and respond to selective pressures. However, it would also exhibit internal fragilities that could be exploited by appropriate therapies targeting the DNA repair machinery. The implications of this hypothesis are discussed.

Keywords: cancer; error threshold; microbial populations; minimal cell; mutator phenotype.

Introduction

In his last book, Mortality, the late Christopher Hitchens wrote: ‘To exist, a cancer needs a living organism, but it cannot ever become a living organism. Its whole malice (…) lies in the fact that the “best” it can do is to die with its host’. In these few words, Hitchens captured the essence of what cancer means in terms of an organised, life-like form that cannot acquire all the traits defining a living being. Because it grows at the expense of the host that generated it, and because it breaks most of the rules that define organismal homeostasis, it expands until no more expansion is possible. The end is shared with its host. But because it occurs through a process of complex adaptations and somatic evolution, we could also say that cancer is more sophisticated, flexible and complex than is assumed in the previous description. Ultimately, when considering the whole picture of cancer as a biological system, multiple views can justifiably coexist.

Cancer populations have been compared to ecological systems [1, 2], parasites [3], viral quasispecies [4, 5], microbial communities [6, 7] or even swarms [8, 9]. Which of these metaphors is best suited? Why so many? This multifaceted view of tumour progression can of course be understood in metaphorical terms as an elephant being touched by a group of blind people: they describe the same entity in very different ways because each of them touches it in a difference place. But the reason for

such multiple views might actually reside in the special status of cancer populations, which inhabit a twilight zone somewhere between the order characteristic of an organised tissue and heterogeneous organisation of changing, microbial populations. Understanding the way in which cancer evolves – adapting in robust ways – as well as its potential weaknesses, requires a consideration of how tumour plasticity emerges and what its limits are. It also requires a better understanding of how order and disorder coexist and the meaning and implications of this coexistence. In this context, cancer cells display a broad array of features (see [10] for a discussion on cancer cells traits) that remind us of the reduced complexity that is characteristic of microbial life forms (which are, by definition, simpler than eukaryotic cells) or even of simple eukaryotic unicellular organisms such as protists [10]. The complexity gap separating unicellular agents – mainly driven by nutrient-limited division – from multicellular ones – characterised by supra-cellular, cooperative traits – can be crossed under stress. It is explored by cancer as it evolves within a given microenvironment, by the immune system when fighting against hypoxia and changing metabolic conditions, and also by some microbial systems thriving under fluctuating conditions.

The potential to overcome selection barriers (both in cancer and microbes) is favoured by a loss of genomic stability. In genomically unstable tumours (virtually all solid tumours display this hallmark), the failure of mechanisms that control the accurate segregation of chromosomes generates a large array of chromosomal arrangements, including losses and gains and a widespread aneuploidy [11]. Such a disordered pattern vividly contrasts with the strict genomic organisation in normal cells. The observation of genetic disorder has usually been associated with high mutation rates in genes that normally function to maintain genetic stability – the so-called mutator phenotype [12–15]. These alterations are caused when stability-related genes (caretakers) are mutated or lost, examples being key genes such as BRCA1, BRCA2, ATM or BLM that are associated with mitotic recombination and chromosomal segregation [16–18]. As a consequence of instability, a very large number of genetic lesions occurs, resulting in a highly heterogeneous phenotype. Because of the great diversity emerging from unstable phenotypes, and despite some success stories, treatments involving cytotoxic drugs directed to specific targets are likely to fail as soon as the selection of resistant clones occurs [19–21].

Generally, the loss of genomic stability and the loss of intercellular organisation might involve a reduction of the cellular complexity, giving place to minimal set of intracellular components able to operate in a robust manner under noisy conditions. In this sense, the similarities between cancer and microbial populations suggests a deep connection between them, which we will explore here. The tendency of unstable cancer cells to cope with stress and selection pressures by losing or down-regulating multiple molecular components must have a limit. The existence of such lower boundaries to cellular complexity is at the core of the conjecture presented here, namely that a minimal construct must characterise evolved, unstable cancer cells. More precisely, we suggest that the loss of multicellular-related traits proceeds until a critical point is approached. A lower complexity limit can be characterised by the presence of: (a) thresholds to stability associated with disordered dynamical processes and (b) structural properties consistent with reduced, critical cellular networks.

Moreover, these two aspects need to be grounded in ecological and tissue levels of description associated with a given microenvironment [7, 9].

Instability thresholds

The loss of regulatory pathways and signalling mechanisms that is associated with genomic instability implies a loss of cellular coherence [7, 15]. This can be noticed at the molecular, cellular and tissue levels, as tumours evolve from benign growth to invasion (Fig. 1A–C). As instability levels grow, tumour cells display increasing levels of aneuploidy, evolving towards a state where replication is favoured over differentiation (see [20] and references therein). This is consistent with a selection pressure for faster cell division and with different traits exhibited by advanced tumours behaving in ways more similar to microbial populations. Such a picture was proposed early on by Nowell [22], who suggested that neoplasm evolution necessarily involves 'a wide array of mitotic variants'. The widespread loss of cellular components associated with differentiation, metabolism, apoptosis, ge nome repair, cell communication, adhesion and motility is driven by a major rewiring and simplification of molecular pathways and control checkpoints. Along these lines, some studies actually suggest that cancer cells might become independent replicators [23, 24], meaning that cells in tumours lose/deregulate a large fraction of cellular components not required for purposes beyond their growth. This picture is in agreement with a well-known, inverse correlation between differentiation and proliferation [1, 21]. If true, we would expect to see cancer cells reverting to unicellular selfishness, as a major transition from a cohesive system to individuality. Support for this idea can be derived from the observation that mutations or loss of genes associated with multicellularity in metazoans appear to be connected to carcinogenesis by helping to promote a reverse transition from pluri- to unicellular traits [25]. As noted in [26], malignant cells are reminiscent of ancient life forms surviving in adverse environments. Evidence for this connection is manifest in ATP-dependent transporter proteins [27], which appear to be up-regulated in cancer cells, and are responsible for multidrug resistance. These proteins are members of a highly conserved super-family of transporters that are capable of pumping out cytotoxic agents [28], and are known to extrude both antimicrobial and antitumour agents from bacterial and cancer cells, respectively. A similar link with the ancient evolutionary past can be found by comparing stress responses associated with increasing levels of genomic instability [6–8,26]. However, unconstrained instability would trigger a widespread degradation of genomic information and jeopardise survival. In this context, excessive instability needs to be avoided in order to ensure viability, whereas low instability would be disadvantageous for the tumour because selection barriers would prevent growth from occurring. Intermediate values would provide the right conditions for stable progression, and it has been suggested that an 'optimal' instability level might exist [29]. This can be verified by considering a simple mathematical model that illustrates both the presence of such optimal instability and the existence of a critical level beyond which the cancer cell population will fail to prosper (see Box 1).

The conflict between reliable cellular behaviour and unstable dynamics defines a tension that reminds us of those physical and biological systems exhibiting phase transitions [30]. In such cases, a conflict between order and disorder makes the system poised in the critical domain separating ordered and

disordered states. One common signature of systems poised at criticality is a high level of diversity and the presence of traits that span broad statistical distributions. An example of such distributions is provided by a pile of sand at criticality [31]: as we continue adding grains of sand to its top, we observe avalanches of sand resulting from colliding grains falling down. Once the pile reaches the maximum (critical) slope, a

41]. It also suggests that unstable cancers might live close to the transition from viable to non-viable. The idea is illustrated in Fig. 2B, where we show how the abundance of mutators in cancer populations would distribute over mutation space. The resulting population will display a huge diversity of phenotypes, including a large number that are ultimately non viable [37, 41, 42].

This tentative picture indicates that, in contrast with normal cells displaying low mutation levels, unstable populations would peak close to the critical threshold, but at some distance from it [41]. A onefold increase in instability would be required to cross the lethality threshold. Because of the intrinsically fluctuating nature of the transition, we should expect, as it is the case, an extremely variable population of (mostly non-viable) cell phenotypes. More importantly, the loss of intercellular organisation suggests an interesting scenario: a reduced cellular complexity that could be mapped into a minimal set of interconnected molecular components operating in a robust way under highly noisy conditions.

Minimal cancer cells?

The concept of cancer cells as minimal replicators raises the question of what the minimal requirements for an autonomous cell are. Current research on minimal genomes suggests that something as small as 500 or even 250 genes would work [43–45]. Recently, Nijhawan et al. [46] shed light on the concept of essential genes for cancer growth describing so-called CYCLOPS (copy number alterations yielding cancer liabilities owing to partial loss). These genes were found essential for tumour cell growth in human tumours. But, generally speaking, what is dispensible? The short answer would be anything that is not on the list of basic components that make replication possible. For a cell within a multicellular organism, the repertoire of housekeeping genes should obviously define a fundamental part of that list. Removable items would include most components related to sensing environmental signals, the preservation of genome integrity and replication checkpoints. Anything that remains should define the minimal cancer cell. Is this cell going to be a robust system? The answer to this question can be addressed via the observation that the selection process pushes cancer cells towards simpler forms of genomic organisation. Support for this idea comes from studies on canine transmissible venereal tumour (CTVT) [47, 48]. The CTVT was first identified in dogs in the late 1800s, when it was found that CTVT could be transferred to new hosts through tumour cells. Murchison et al. have recently provided a whole-genome sequence of CTVT from two different tumours. Interestingly, together with the observation of massive mutations accumulation, they reported the loss of 646 genes that may be dispensable for survival and proliferation of somatic cells [47]. The work of these authors demonstrated the large-scale evolutionary fate of these tumour cells, resulting in highly robust mammalian somatic cells able to survive for millennia despite a massive mutation burden and gene loss.

If cancer cells lose parts of their cellular machinery as they become unstable, further instability builds up. The sharp transition that is observed once a cell crosses given instability levels – which is well known in RNA viruses – was used early on to develop potential therapies based on lethal mutagenesis. These – in the form of promutagenic nucleoside analogues – were successfully tested in vitro, and shown to impair viral replication of HIV [49]. They have also demonstrated proof of principle in other contexts [50].

As we suggested above, the similarities between unstable cancer and RNA viral quasispecies suggest that thresholds of stability might also be present in carcinogenesis [51]. This is consistent with the increasing emphasis placed on targeting DNA repair pathways as a key part of the strategy against cancer, specifically, combined with other drugs [52–55]. The hope of most cytotoxic, anti-cancer therapies is, in one way or another, founded on the possible existence of some sort of 'Achilles heel' that can be identified in a reliable way; indeed, in some cases such targeted therapy is very effective. Interestingly, in cancer itself, the term 'Achilles heel' has multiple faces: these include molecular targets for specific genes such as p53, telomerases, cellular processes such as autophagy, or ways of managing tumour vasculature and metabolism. However, most traditional approaches to this problem have failed to incorporate the intrinsic evolutionary nature of tumours [1, 20, 52]. The identification of an Achilles heel in cancer demands a well-defined molecular interaction map of cancer cells. If it truly exists, and if unstable cancer cells share a backbone of key genes and pathways that need to be maintained, then it should be possible to identify a minimal gene complement of cancer cells for each type of tumour. More precisely, since genes interact, and interactions are ultimately responsible for the phenotype, we could think in terms of the minimal cancer cell network and its associated stable expression patterns or attractors.

The theory of a cancer network attractor was proposed early on by Kauffman [56] in the context of Boolean network models. In a nutshell, a gene regulatory network is represented by means of a web of molecular interactions involving both activation and inhibition, and displaying a finite number of stable states defined as a specific expression pattern [57]. Each of these patterns can be identified in terms of a cell type. Cancer progression implies a disruption of cellular pathways, and thus it seems reasonable to speak of cancer attractors [58, 59]. Their presence offers a promising, systems view of carcinogenesis, allowing interpretation of genomic data via a network perspective [60, 61]. Moreover, the stability of such attractors and the discrete nature of their associated phenotypes would result from the existence of robust rules underlying the 'epigenetic' landscape, as defined by Waddington [62, 63]. Two aspects of such landscapes are important here: the first concerns ways that such a landscape is explored through carcinogenesis; the second is the question of how such exploration affects the attractors themselves. If cancer cells result from a drastic loss of the original network organisation, what type of landscape should we expect to observe?

If a minimal construct exists, then we would have a map of the relevant actors in cancer progression and how the cancer landscape is explored. In particular, the tendency towards an increasingly unstable state surely facilitates an expansion through genotype space. A simple model of cancer development with cells carrying a digital genome (Fig. 3A–C) reveals a complex, emerging web of

paths connecting different genotypes [64]. Within a virtual tumour, each cell has something that resembles a genome that includes replication and stability-related genes (responsible for maintaining genome integrity) as well as housekeeping genes. Starting from a homogeneous population of cells, spontaneous mutations can arise, leading to mutated genes, which sometimes are stability-related genes. Figure 3D and E shows an example of the simulation results revealing a network connecting different genotypes explored by the growing tumour over time. If we measure different statistical traits of these virtual tumours, power laws are found that are consistent with the critical nature of the population dynamics. More common digital genotypes having higher populations are represented as larger spheres. The majority of states are instead occupied by a small number of digital genomes. These differences suggest an interpretation in terms of driver and passenger genes [65], but that could be an oversimplification. Combinations of mutations would, instead, define driver and passenger genetic clusters. This view is consistent with an evolutionary perspective of cancer founded on clustering, altered genes based on gene family membership or affected signalling pathways [66–68]. Moreover, as discussed in [64], the observed genotype network is highly heterogeneous in its pattern of pathway interconnections, with a high variance that might make average values meaningless. Here too, power laws are common, and most genotypes are linked to just a few single-bit mutant neighbours, a few of them having many neighbours. Interestingly, there seems to be only a weak correlation between the fitness (growth rate) of a given genotype and its population occupancy. The multiplicity of paths is a direct consequence of the unstable dynamics. The presence of mutations in mutator genes seems to create the appropriate conditions for a neutral or quasi-neutral exploration of sequence space [40]. Once again, as occurs with true minimal replicators such as RNA viruses, cancer cells might gain advantage in terms of evolvability by losing regulatory complexity and acquiring phenotypic variation by means of increased population heterogeneity. Although the model is an oversimplification, it suggests that the scaling laws reported from cytogenetic and molecular studies might point towards a critical (or near-critical) state.

Bridging space, ecology and epigenetics

Dissecting the cancer web and establishing a minimal set of components is not only a way of looking for a minimal cellular network: it is also a way to find the real Achilles heel of the disease. Single genes or proteins might be targeted effectively by a well-designed drug, but perhaps the ultimate solutions are hidden inside cellular pathways. In our search for the minimal cancer cell, we might gain some knowledge by looking at the minimal architecture of cells in a tumour. Such cells become minimal through an evolutionary process involving rapid replication, loss of cell communication and escape from recognition by the host immune system. What remains intact, either at the cellular or at the population level, would trace the boundaries of the minimal system. Our

conjecture is based on the seemingly inevitable increase of instability until populations approach levels that can compromise their viability. By reducing the limitations associated with growth control and the maintenance of the multicellular state, cancer cells lower their internal complexity while entering into a disordered phase.

Knowledge of the network organisation of the cancer web would provide invaluable insight into the disease and its progression. It could give us a map of the cancer world. The question thus might be stated as follows: Is there a minimal construct that is capable of encapsulating the complexities and fragilities associated with unstable cancer cells? In previous sections, we have considered a number of analogies with microbial or viral populations and the characteristics of unstable cancers. Different observations support the picture of unstable cancer cells as minimal replicators. Some additional components must be taken into account in order to complete our picture:

(1) The spatial dynamics of solid tumours result in a population of diverse clones coexisting together within a tissue. This coexistence is largely a consequence of the decreased impact of competitive exclusion arising from the limited range of effective cell-cell interactions. The situation is describable in terms of a metapopulation [69] where multiple subpopulations of weakly connected subclones coexist. Spatial diversity translates into an accumulation of clonal diversity that has been shown to predict progression in adenocarcinomas [70]. Space strongly influences population dynamics, allowing for the coexistence of competitors when no such coexistence is possible in a full, mixed scenario [71], hence increasing the latency time for cancer [72] and relaxing the error threshold [73].

(2) We are assuming that the ecological behaviour of a cancer population is mainly determined by competition among nearly independent replicators. However, differ ent cancer cells might actually cooperate [74]. More generally, we could think of the tumour as the union of species-specific, cellular networks exploiting available resources, perhaps not far from some microbial interspecies networks [75]. An example is given by biodegradation networks [76] where the actual web is the integration of all possible pathways implicated in the degradation of pollutants. The result of such integration departs from the minimal cancer cell. Instead, an ecosystems-level perspective of the tumour as an ecological community should be considered [1, 77, 78]. In that case, targeted therapies would require considering network fragilities that are closer to those found in ecosystems [79, 80]. Perhaps, more interestingly, the existence of several coupled networks operating on different scales, which can easily exhibit abrupt transitions, might improve the search for fragilities [81, 82].

(3) High instability has an important impact on the potential set of steady states that are available to the tumour. A minimal cancer cell would be characterised by one or a few well-defined potential attractors (Fig. 4A). However, increasing instability can deeply deregulate the epigenetic landscape as a consequence of modifications in chromatin structure [83, 84], thus making attractors much less stable (Fig. 4B). Existing models of gene regulatory networks indicate that increasing levels of noise make different potential states much more accessible to each other [85, 86]. Such a stochastic view of the cancer landscape is consistent with a picture of carcinogenesis that is much less predictable than the one expected from deterministic genetic pathways [22] and might hide a great potential for adaptation.

Discussion and prospects

The stochastic dynamics displayed by unstable cancer cell populations are the ultimate cause of their multifaceted organisation. While normal cells avoid mutations through a highly accurate replication process, cancer cells require the accumulation of mutations activating oncogenes, inactivating tumour suppressor genes and avoiding immune system responses and other forms of organism-level selection barriers. As a consequence, tumours are heterogeneous assemblies that are composed of cells that have become simpler. Such simplicity is marked by a tendency towards unicellular traits that include a reduction of functional complexity and an increased deregulation favouring proliferation over tissue order. On the other hand, the loss of cellular functions in favour of replication (through the loss of key players that control the cell cycle) is constrained by the local microenvironment and the intrinsic properties of the underlying morphogenetic landscape. Instability has optimal values, but perhaps more importantly, critical boundaries. The structural patterns of genomic disorder (as given by aneuploid karyotypes) and the dynamical behaviour of cancer populations should be seen as two faces of the same coin indicating the proximity towards critical points. In this sense, instability thresholds probably exist, and lethal mutagenesis should be expected once a cell crosses them. The complex nature of carcinogenesis is a consequence of the entangled nature of the molecular networks involved. They are indeed far from well understood. It could be argued that, because of the disordered nature of unstable tumours, our battle against cancer might be much harder than expected by the pioneers of cytotoxic drug treatments. Indeed, cancer continues to be a major problem, largely remaining as incurable today as it was half a century ago. The fact that tumours become similar to microbial communities and respond collectively and flexibly to external stress calls for novel therapies that take into account Darwinian evolution [1, 19, 20, 87]. Looking at cancer replicators in terms of regulatory and signalling networks [88– 90], particularly in relation to genomic instability [91], seems a promising strategy. In this context, we should not forget that theoretical studies on complex networks have revealed a number of potentially universal traits, power law patterns and fragility to targeted damage being two of them. As genomic instability appears to be a hallmark of cancer, so might the fragility of the cellular networks involved.

Identifying the minimal cancer network should be the first route of validation of the conjecture suggested here. Several key steps could help to test our hypothesis:

(1) Cancer cell networks should display a large variety of perturbations, to be detected as a large variability in the connections among genes; but it should also be possible to study the core of genes that appear to be required to sustain cell viability. Gene inventories are a first step. Indeed, such an extremely high degree of genomic heterogeneity, observed on multiple scales, casts serious doubts on the statistical significance of so-called ‘average profiles’. Available evidence from the analysis of cancer networks indicates the enrichment of some given structural motifs, but these webs tend to be dense, and it is difficult to extrapolate meaningful patterns of organisation. A proper approach to cancer requires using available methods that permit complexity reduction to a smaller subset that can represent the ‘kernel’ of the cancer genome. This approach [92–94] has been followed by some authors in search of a logical or functional core of molecular control units. They all involve a systematic reduction of network complexity that retains only the skeleton associated with those

gene-gene interactions that are essential. A similar approach should be taken with existing cancer network data sets.

(2) Unstable tumours that are poised close to a critical point should display characteristic patterns of fluctuations. Additionally, the approach to the critical regime is expected to display dynamical trends that are used in ecology as warning signals indicating potential regime shifts [95]. Such trends can be detected by means of measurable traits in some pathological processes [96] including cancer. Further work should take advantage of tumour heterogeneity (in both space and time) through appropriate ecological measures of diversity [97] to validate the criticality hypothesis. Previous work on the progression dynamics of adenocarcinomas [98] supports the use of such diversity measures for characterizing and predicting tumour progression.

(3) Two main aspects of the minimal construct hypothesis have been presented above. Determining how structural (network level) and dynamical patterns are connected requires a theoretical framework that is able to establish the links between pattern and process, and ultimately make testable predictions. Despite the considerable and increasing attention that is being given to mathematical

and computational models of cancer progression [99–102], an important piece of realism is missing from the field: an explicit consideration of chromosomes. With few exceptions [32, 103–105], models of tumour progression largely fail to incorporate this trait. The complexities associated with incorporating these higher-level structures above the gene level introduce a challenge in modelling, but the reward is great. There is extensive literature on quantitative genetics concerning cancer karyotypes that a consistent model should be able to reproduce. As a source of potential validation, the available information concerning chromosomal changes in time and space is vast and detailed.

Conclusions and outlook

A life-history perspective of unstable cancer provides meaning for the apparently paradoxical resilience of tumours displaying genomic instability. As pointed out in [10] tumourigenesis consists in prioritizing cell-level survival over the multicellular order characteristic of the metazoan phenotype. In this context, it seems reasonable to conjecture that genome reduction takes place in concert with the somatic evolution of unstable cancer cells, resulting in some type of minimal construct. If the description of cancer as a species is correct [106] the idea of a minimal cancer cell network should also be testable. But it is not like anything in biology that we could use for comparison. By losing essential parts of its molecular machinery and reverting to a unicellular-like state, the tumour effectively experiences a ‘time reversion’ [107]. It somewhat reminds us of Benjamin Button, the famous character in Scott Fitzgerald’s short story: always younger, losing its memory and becoming closer and closer to an immature state [58]. Nothing like that appears to occur in any other biological system. We need to make sense of this unique pattern.

We are starting to unravel the molecular maps describing the complexity of cancer. Dedicated efforts have been made using high-throughput methods to build a comprehensive picture of the disease [108]. However, as more and more data pile up, the picture becomes more and more complex.

Interpreting and understanding these cancer genomes might strongly benefit from well-defined theoretical models (or hypotheses) such as the one proposed here. The ongoing efforts already reveal patterns of order within genomic chaos. Such order provides us with a glimpse of different cancer networks related to different cancer types, but we are still far from understanding their functional meaning. The minimal cancer hypothesis might offer such a unifying framework.

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Figures

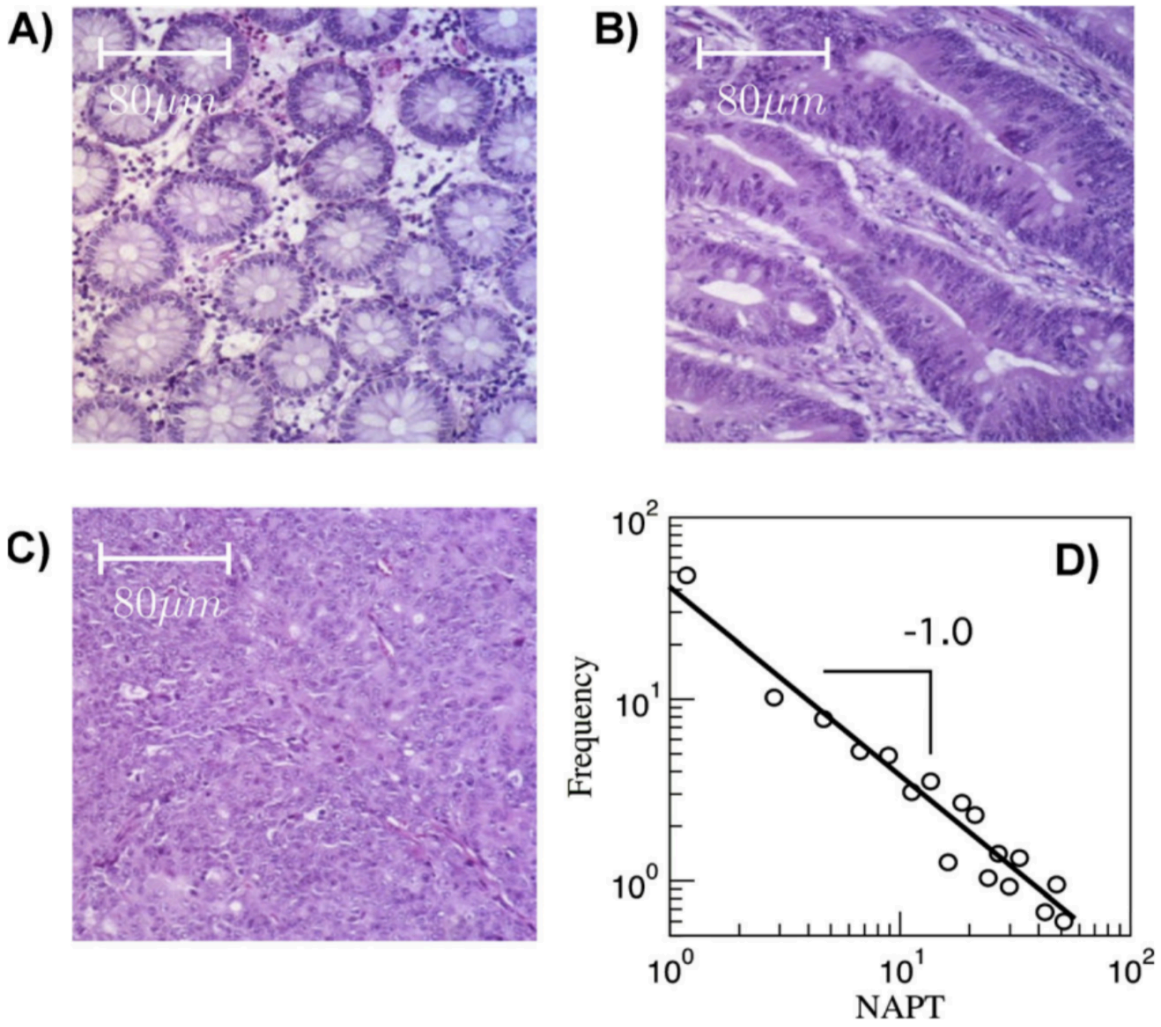

Figure 1. A typical progression (A–C) towards disorder in colon cancer is illustrated here for three different stages of colon cancer. Here, we have A: normal tissue, B: dysplasia and C: undifferentiated, invasive colon cancer (pictures courtesy of Bob Gatenby). Statistical analyses of such unstable populations reveal very broad distributions of chromosomal aberrations, as shown in (D) where we plot the number of these aberrations per tumour (NAPT) in colorectal carcinomas (redrawn from [31]). The frequen cy F(NAPT) follows a power-law decay F(NAPT) = 1/NAPT.

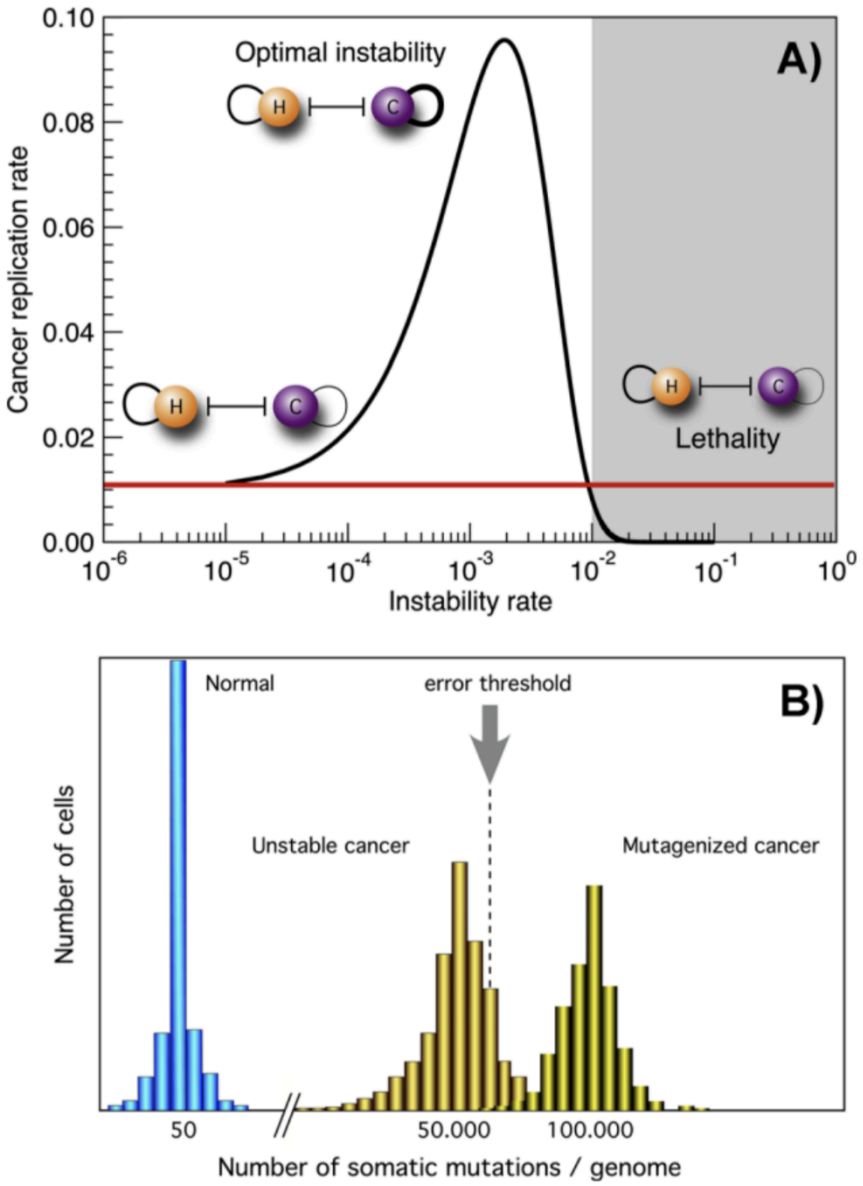

Figure 2. A: Trade-offs between advantageous mutations that hit growth-related genes and deleterious effects associated with the damage of housekeeping genes (see Box 1). The function defining the increase in replication rate in cancer cells having an instability level of m is shown here. As predicted by the theory, the optimal instability rate occurs around the inverse of the number N_h of housekeeping genes, i.e. $m_{opt} = 1/N_h$ (here, we use $N_h = 500$). For levels higher than optimal, a boundary is crossed separating viable from non-viable cancer populations. A more statistical picture of the effects of mutagenesis in cancer is displayed in (B). Normal cells have very small mutation rates, measured in terms of the number of somatic mutations per genome, whereas in cancer cells exhibiting the mutator phenotype, the distribution would broaden and shift towards high mutations, probably close to the error threshold. If that picture is correct, additional increases of mutation rates would further shift the population beyond the edge of viability. Adapted from [40].

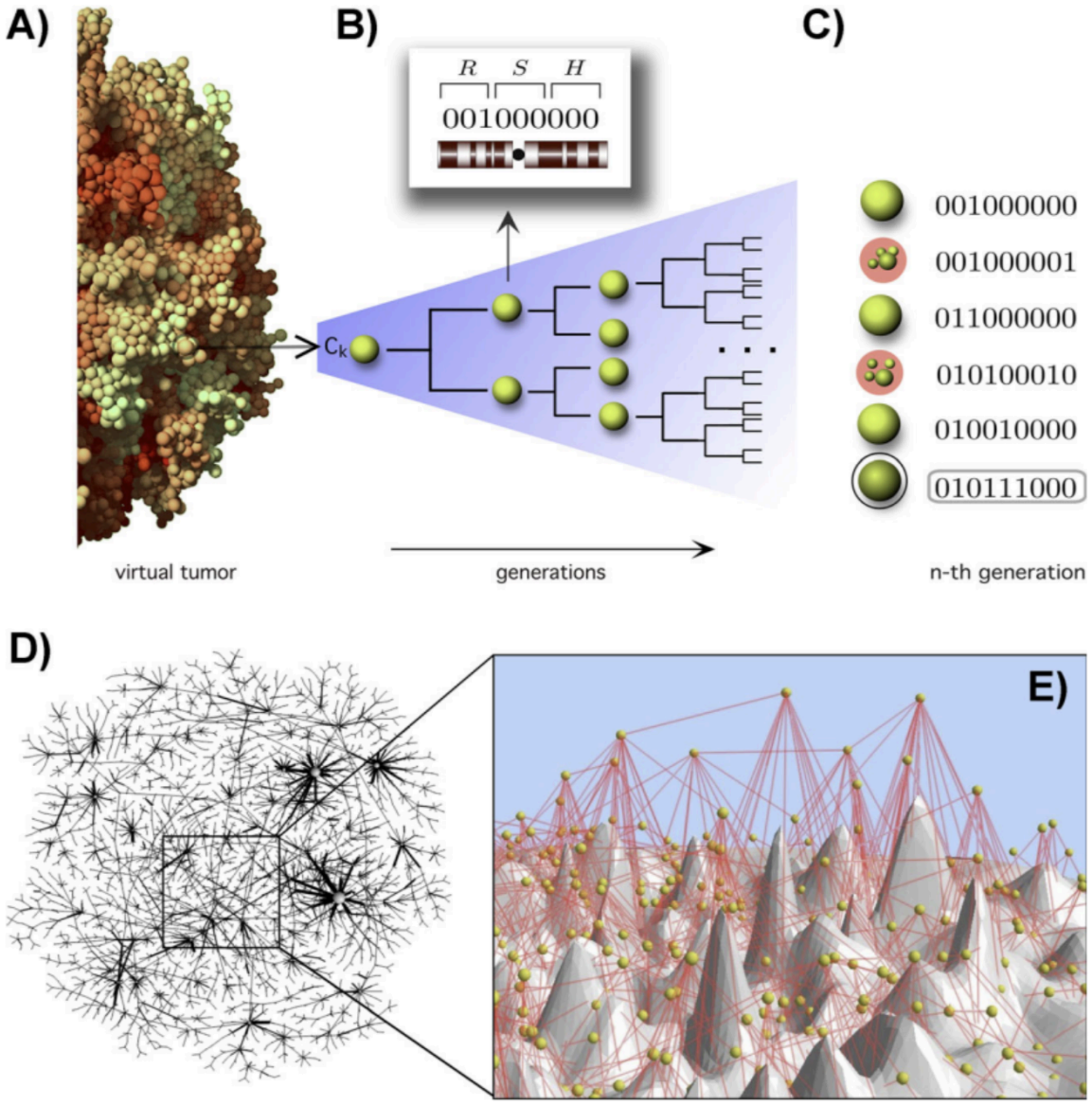

Figure 3. The evolutionary dynamics of cancer cells can be simulated with digital genomes represented by binary strings. Each cell in a virtual tumor (A) contains a digital genome, here represented as a 9-bit genome with three genes for each class, namely replication (R), stability (S), and essential, housekeeping (H) genes (B). After n generations, (C) cells can accumulate mutations affecting different traits. Tumour cells with accumulated mutations in the R group will replicate much faster. Tumour cells with mutations in the S genes will have higher mutation rates. Cells with mutations in housekeeping genes will die. The circled cell at bottom right contains several mutations in the S group and will easily generate a progeny with lethal changes. In general, each string can mutate into a different one, and such mutation is indicated as a link connecting the two genomes. The resulting network (D) provides a picture of the unstable landscape explored by the evolving population. We display the network of different mutant genomes and their single-bit neighbours. A zoomed subset is displayed in (E), where the fitness associated with each local string is indicated by the height of the fitness landscape.

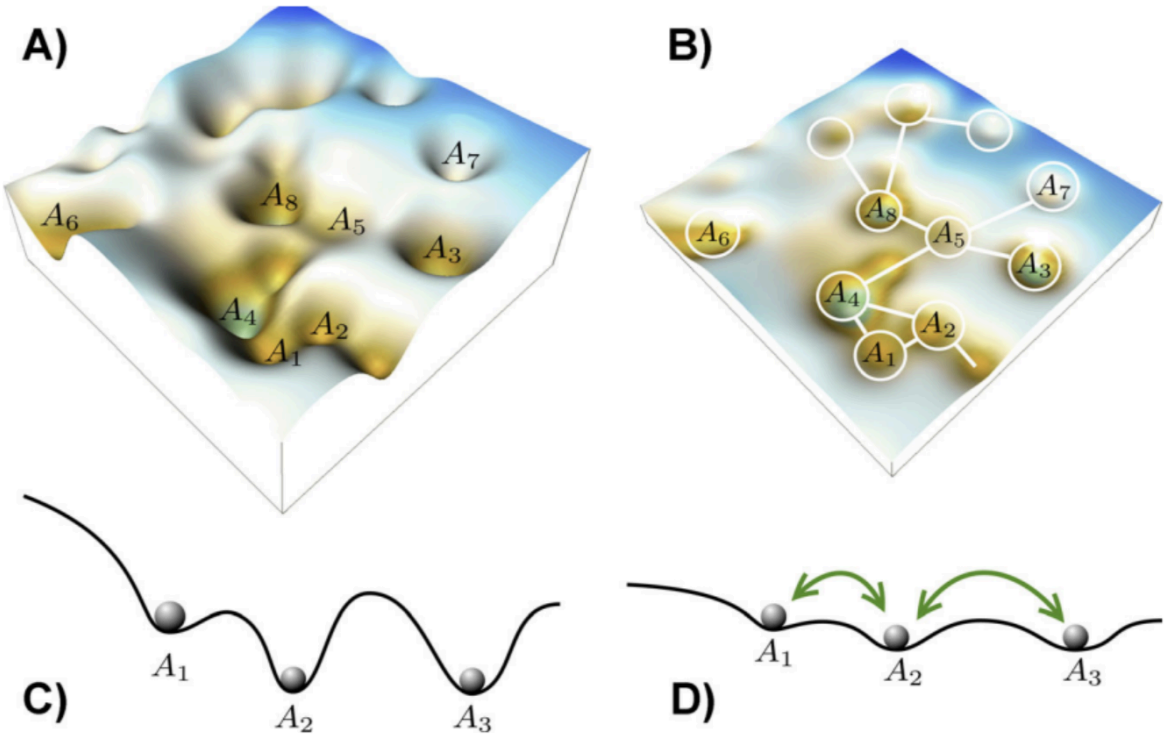

Figure 4. Phenotypic landscape of a complex tissue and its change under genomic instability. A: A hypothetical landscape where each valley would correspond to some ‘attractor’. Attractors are stable states reached by the system under ontogenetic processes, and stabilised through appropriate feedbacks and regulatory interactions. Small, stochastic perturbations will (typically) be unable to move the system from its ‘resident’ valley. However, under high instability levels, the landscape becomes flatter (B) and transitions between attractors become more likely. This is illustrated in (C and D) by means of a one-dimensional plot in which we can better appreciate the impact of making the landscape flatter as a consequence of instability. The barriers between minima are now easier to cross if fluctuations are present.

Box 1

Optimal and 'critical' instability rates

Assuming that two populations of both normal and cancer cells are at play, we will introduce a simple model of cancer instability. The model assumes two homogeneous populations of cells, and thus no cell variability is allowed in each compartment. If we indicate as rn and rc the rates of growth of normal and cancer cells, respectively, we can write

$$\frac{dN}{dt} = r_n N - N\phi(N, C)$$

for the host population and similarly we have:

$$\frac{dC}{dt} = r_c C - C\phi(N, C)$$

for the cancer population.

We can assume that the growth rate r_c of the cancer cell population depends on the instability m of such population. Here, *m* will be a probability. For low m, we should expect to observe an increase in the growth rate because growth-related genes will have been hit. If we indicate as N_r the number of such genes, we can guess that the growth rate will increase due to such events as

$$f_1(\mu) = r_n + \mu N_r \delta r$$

where *d* gives the increase in growth for each hit. Similarly,we should expect a decrease in the growth rate due to the potential damage produced if a housekeeping gene is damaged or lost. If N_h indicates the number of such genes, the probability that no single one is damaged will read

$$f_2(\mu) = (1 - \mu)^{N_h}$$

and thus the final rate of replication will be the product:

$$r_c(\mu) = f_1(\mu) f_2(\mu) = (r_n + \mu N_r \delta r)(1 - \mu)^{N_h}$$

It is easy to see that this function has a maximum at a given optimal instability rate. An example is given in (Fig. 2A) where we plot rc for a given combination of parameters. It is possible to show that the maximum is achieved at an optimal instability level

$$\mu_0 \approx \frac{1}{N_h}$$

and this is actually a prediction of the model: unstable tumours should approach an instability level that scales with the inverse of the number of housekeeping genes.

The competition between both populations is introduced through the function $\phi(N, C)$. If we consider that the overall cell population *C + N* is constant (because cells fill a given fixed space), then we have *d(N þ C)/dt = 0 = dN/dt + dC/dt*, and thus we can reduce the system of two equations to just one. If we use *N + C = 1* (normalised population), the function $\phi(N, C)$ reads $\phi(N, C) = r_n N + r_c C$, which is actually the average rate of growth. It is possible to see that the equation describing the dynamics of the cancer cell population is now

$$\frac{dC}{dt} = r_n(\Gamma(\mu) - 1)C(1 - C)$$

Two fixed points are present: the zero-population one, i.e. *C = 0*, and the maximum population state, here, *C = 1*. The fixed point *C = 0* is stable if $\Gamma(\mu) < 1$ and unstable otherwise. By properly defining the function, we might be able to define the conditions under which genetic instability allows cancer growth to occur and overcompete the host tissue. The critical mutation rate separating the two scenarios is sharp and defines a phase transition.