OPINION

Cancer as a robust system: implications for anticancer therapy

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Cancers are extremely complex, heterogeneous diseases. Many approaches to anticancer treatment have had limited success — cures are still rare. A fundamental hurdle to cancer therapy is acquired tumour 'robustness'. The goal of this article is to present a perspective on cancer as a robust system to provide a framework from which the complexity of tumours can be approached to yield novel therapies.

Robustness is the ability to maintain stable functioning despite various perturbations. Complex systems are successful if they are robust against a wide range of external and internal stresses. Specific examples of traits that are used in biology to maintain robustness include adaptation (to external cues), tolerance of stochastic fluctuations in the kinetics of protein–protein interactions and in protein concentrations, and tolerance of stochastic noise[1–3]. Typically, these kinds of robust features are enabled by feedback controls, redundancy (such that functionally equivalent or functionally overlapping modules can substitute for each other), modularity (which facilitates physical or logical separation of subunits, thereby preventing spread or amplification of local perturbations) and structural stability[4,5]. One of the features of robust systems is a gradual degradation of function in response to damage, although interference with strategies for robustness can lead to catastrophic failures.

Robustness is increasingly recognized as being a conserved organizing principle in biology, as highlighted by recent systems-level studies of bacterial chemotaxis[6–8]; the cell cycle[10]; circadian rhythms[10,11]; *Drosophila* segmentation[12,13]; tolerance of stochastic fluctuations in fundamental biochemical processes — such as transcription and protein interactions — due to the small numbers of molecules involved[3,14] and point mutations in promoter regions[15]; and large-scale biochemical networks[16,17]. Not only does robustness maintain homeostasis in complex organisms, but the machinery that maintains robustness can be hijacked to maintain dysfunction, as occurs in tumour resistance to anticancer drugs.

The concept of robustness (or fragility) can be more formally defined in terms of a system with a property that is maintained in the presence of specific perturbations. The mechanisms and components of the system that are responsible for this robustness can be quite varied and complex, and often create fragilities for other properties and perturbations. As an analogy, in aviation, the autopilot (a component) uses feedback control (a mechanism) to robustly regulate the flight path (a property) of an aircraft (a system), despite displacing forces on the vehicle owing to variable atmospheric conditions. It also uses redundancy to provide robustness in response to sensor, actuator and computer-component failures. The resulting complexity can create novel fragilities that are not present in more primitive technologies, such as system failures due to software bugs. The trade-offs between resource cost, complexity, robustness and fragility dominate the design of many engineering systems, and might also be important in cancer biology.

An organism is a system that is robust against a broad range of perturbations, but is fragile in terms of developing cancer. At the same time, cancer can be viewed as a robust system that is composed of tumour cells[18] — the proliferation of these tumour cells is a property that needs to be maintained, and various anticancer therapies and naturally occurring microenvironmental and immunological responses are perturbations that are imposed on the system. When we refer to the robustness of cancer, we mean the robustness of cancer as a system, rather than that of an individual tumour cell. There is great interest in exploring the mechanisms of this robustness of cancer as a system in order to be able to reduce it. In addition, the accompanying fragilities might offer the potential for novel therapies.

How is robustness exploited in cancer?

Tumours are highly robust and maintain their proliferative potential against a wide range of anticancer therapies. Two aspects of robustness are exploited by tumours — functional redundancy, which is enabled by cellular heterogeneity, and feedback-control systems that are used to facilitate survival in hazardous environments (for example, due to anticancer drugs or hypoxia).

Functional redundancy. In general, tumours maintain cellular heterogeneity, which means that the survival of a subpopulation of cells with metastatic potential after anticancer therapy can lead to tumour recurrence[19–24]. Heterogeneity facilitates robustness through redundancy, and subsystems that are killed by chemotherapy can be functionally replaced to ensure tumour proliferation and survival. Robustness through redundancy occurs at two levels. First, multiple copies of identical components provide a storehouse of available parts when some are lost. This type of redundancy, which can be referred to as homogeneous redundancy, is simple and effective when hostile stimuli are localized — that is, when they are targeted to a specific component. However, if all susceptible components are

exposed and successfully targeted, they may all fail, in what is known as common-mode failure. If, however, redundancy is mediated by functionally equivalent but heterogeneous components (known as heterogeneous redundancy), a system is less likely to experience common-mode failure.

Both kinds of functional redundancy are present in advanced flight-control computers and they are crucial to aircraft operation. Three or more computers implement identical control algorithms (homogeneous redundancy) and make a majority decision to detect and ignore failed components. The computer hardware and software, however, are otherwise made as different as possible (heterogeneous redundancy) to avoid common-mode failures. Creating this combination of homogeneous outcome and heterogeneous implementation is a major challenge in engineering design.

The essential robustness of tumours is attained through heterogeneous redundancy. Extensive cytogenetic analysis indicates intratumoral heterogeneity[25–28], as well as the heterogeneous, often non random, spatio-temporal distribution of genetically heterogeneous tumour cells[29–31]. It is important to recognize that the robustness of a tumour as a system is not equal to the robustness of individual tumour cells. Even if each tumour cell is more fragile than a non-tumour cell in response to a particular chemotherapeutic drug, heterogeneous redundancy can give rise to robustness at the system level through genetic variability in the pattern of drug resistance. In addition, intratumoral heterogeneity should not be confused with intertumoral heterogeneities that define different tumour characteristics in individual patients. Genetic instability is the cause of intratumoral heterogeneity and this leads to various genetic alterations[32], which include mutations that cause only small changes in sequence, gene amplifications, chromosomal translocations[33,34] and aneuploidy[35–37]. For the sake of simplicity and readability, in this review, 'mutation' is used to indicate genetic transformations that are caused by any of these possible mechanisms, unless otherwise specified.

Despite the recognition that genetic heterogeneity is a major cause of acquired drug resistance[38,39], many anticancer drugs are mutagens that could potentially cause *de novo* drug-resistance mutations. Unless a therapy can completely eradicate tumour cells and tumour stem cells[40], disease recurrence is possible. The complete eradication of tumour cells is, as we know, often unfeasible, particularly in solid tumours of internal organs. In some aspects, intratumoral heterogeneity is similar to the heterogeneity seen in viruses, plague-forming quasi-species of bacteria[41] and insects that have resistance to a diverse range of chemical agents and antibiotics. Host–tumour dynamics, just like host–parasite dynamics, entails co-evolution under the selective pressure that is imposed by host environments, including antitumour drugs. Intrinsic genetic instability, as well as mutations that are caused by antitumour drugs, contributes to maintaining and increasing the heterogeneity of tumour cells that is essential for tumour evolution. Taming this inherent heterogeneity is the main challenge in the development of effective anticancer therapies.

Feedback-control systems. Multiple layers of feedback loops and associated gene-regulatory events are involved in the robustness characteristics of tumours at the levels of intracellular and tumour–host interactions. At the cellular level, feedback controls can give rise directly to robustness against chemotherapy. For example, tumour cells that turn on the expression of the multidrug-resistance 1 (*MDR1*) gene acquire multidrug resistance by exporting drugs out of the cell through an ATP-dependent efflux pump, P-glycoprotein (P-gp), which is encoded by *MDR1* (REFS 42,43). This is a simple, but effective, feedback-control mechanism to minimize cytotoxin levels. Another example is tumour overexpression of MDM2, which causes degradation of p53, effectively blocking apoptosis[44,45]. The MDM2–p53 interaction functions as a negative feedback loop to maintain optimal levels of p53, and also creates certain dynamics (pulsed or oscillatory) of p53 expression levels — instead of sustained expression — after serious DNA damage[46]. These are examples of feedback controls that are increased by mutations associated with tumour progression, but other feedback-control mechanisms also contribute to robustness. The cell cycle, for example, is considered to be robust against a certain level of perturbation, because of several feedback-control loops[9]. These loops can contribute to tumour robustness by preserving proliferation potential, even after chemotherapy.

In addition to intracellular feedback loops, tumour cells activate feedback loops in response to their environment, which initiates events that improve the environment. Complex, multilayered and multidirectional interaction loops occur between tumour cells and the stroma and extracellular matrix, immune cells, the vasculature and other tumour cells[47]. Solid tumours initiate responses to innate tumour-suppression mechanisms in the environment, including polarization[48]. When tumour growth is not balanced by vascular growth, hypoxic conditions result, initiating a cascade of responses that allow the tumour to cope with its environment[49–54].

When the environment is not optimal — for example, in cases of nutrient or oxygen deprivation — tumour-cell survival can be assured by adaptive changes, responses that change the environment, or migration to a new environment (FIG. 1). Tumour cells take advantage of all of these strategies.

How can we control robustness?

One reason for understanding tumours as robust complex systems is to adapt systems-level analysis and the control of systemic robustness to the development of anticancer therapies. Given that a diverse range of mutations confers redundancy and robustness, this concept suggests that reducing heterogeneity is a logical primary strategy of anticancer drug therapies. This model also indicates that if the reduction of heterogeneity is not possible, then an alternative strategy is to maintain heterogeneity and aim to trigger slow — rather than rapid — regression of a tumour. Either way, the priority is that an increase in heterogeneity must be avoided.

Reducing heterogeneity. The active reduction of heterogeneity implies that chemotherapy kills all tumour cells, except those that contain a specific genetic feature. The surviving cell population is then, by design, more homogeneous as a target for a second anticancer drug that specifically targets the characteristic genetic feature. Third-line chemotherapy can similarly be chosen to target a specific homogeneous genetic feature.

Superficially, this approach — which is based on the sequential use of different drugs — seems similar to a treatment strategy inspired by the Goldie–Coldman hypothesis, in which two non-cross-resistant treatment regimens are used[38,55]. Clinical testing of this approach gave mixed, but largely unimpressive, results[56,57]. However, a careful analysis of the Goldie–Coldman hypothesis indicates that the simple use of two regimens that do not cause cross-resistance is insufficient to achieve the predicted results. The hypothesis implicitly assumes that a mutation that causes resistance to a drug occurs only when that drug is used, and that this effect is dose-dependent. However, if the probability of a mutation occurring that makes a cell resistant to a drug is independent of the dose of the

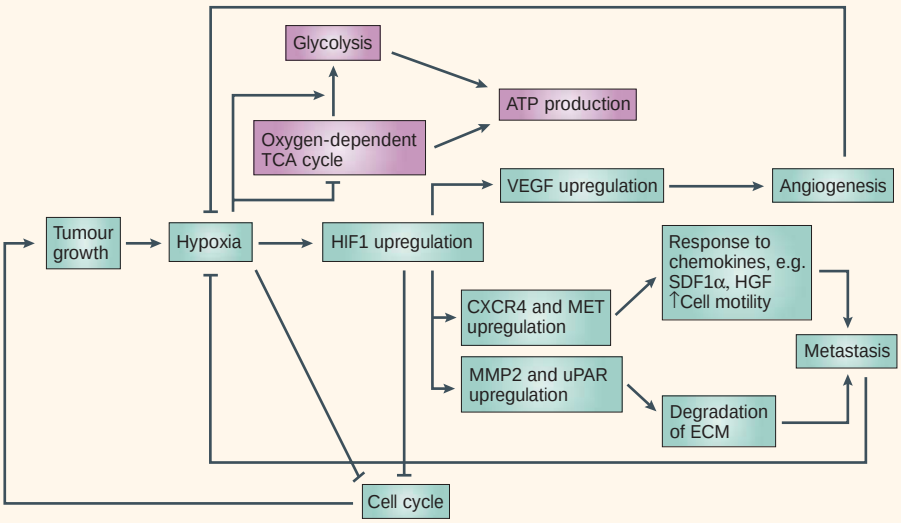

Figure 1 | **Feedback loops for hypoxia responses of tumour cells.** Hypoxia occurs because of a rapid increase in tumour mass that outpaces angiogenesis. Hypoxia induces hypoxia-inducible-factor 1 (HIF1), which upregulates various genes, including those that encode vascular–endothelial growth factor (VEGF)[116–118], CXCR4 (REF. 119), MET[120], matrix metalloproteinase 2 (MMP2), and the urokinase-type plasminogen-activator receptor (uPAR). At the same time, progression through the cell cycle is inhibited by HIF1-dependent and -independent mechanisms. VEGF upregulation promotes angiogenesis, so that hypoxia of tumour cells can be resolved by vascularization. Simultaneously, chemokine receptors such as CXCR4 and MET are upregulated, so that tumour cells can respond to chemokines in the environment. MMP2 and uPAR are upregulated, leading to degradation of the extracellular matrix (ECM), so that tumour cells can migrate away from the hypoxic region and metastasize[120,121]. When hypoxia is resolved in this way, cell-cycle arrest is released and further proliferation is initiated. Multiple feedback loops ensure robust responses of tumour cells to hypoxia. In response to nutrient deprivation, tumour cells can also switch metabolic pathways from an oxygen-dependent tricarboxylic acid (TCA) cycle to glycolysis — both of which result in ATP production[122]. Mechanisms that maintain tissue integrity despite changes in oxygenation are hijacked by tumours to ensure tumour progression and survival. Correcting this hijacked mechanism has been proposed as a means of anticancer therapy[48,123–126], and this might be effective if potential heterogeneous feedback can be fully controlled. HGF, hepatocyte growth factor; SDF1α, stromal-derived factor 1α.

drug, it is possible that cells that survive treatment with one drug have already acquired a mutation that makes them resistant to the second drug. So, tumours eventually acquire resistance to multiple drugs, and this explains clinical failure.

An approach that is aimed at the control of robustness imposes stronger constraints than the Goldie–Coldman hypothesis: if multiple drugs are used sequentially, cells that survive one drug have to be vulnerable (or at least non-resistant) to the next drug that is used. Each drug must have the ability to impose independent selective pressures to acquire mutations, so that only cells with a specific mutation pattern survive.

The challenge of designing drugs that impose these pressures is enormous, but recent lessons from studies using novel chemotherapeutic agents indicate that the strategy has merit. Clinical experience with imatinib mesylate (Glivec) both demonstrates the usefulness of targeting tumours that have well-understood molecular signatures, and also provides preliminary data to explain how resistance emerges when specific molecular targets are inhibited. Imatinib inhibits the oncoprotein ABL, and is very effective for early-stage chronic myelogenous leukaemia (CML), but not for CML at an advanced stage, such as blast crisis[58]. Gorre *et al.* reported that patients who have complete haematological remissions and then relapse have specific mutations in the *BCR–ABL* translocation region[59]. This observation is the subject of debate[60,61], and the conditions that give rise to such selective mutations are unknown. However, if cells that survive imatinib therapy are (relatively) homogenous for such mutations, or are at least enriched for a point mutation, this might mean that heterogeneity could be actively reduced by the use of a specific drug. The treatment of heterogeneous tumour populations can therefore generate genetic variants with reduced heterogeneity, which escape environmental and therapeutic constraints to proliferate further. Heterogeneity can quickly increase again due to genetic instability.

Inducing dormancy. The second strategy that is suggested by the systems-level analysis of cancer as a robust system is to induce dormancy, meaning a state in which the size of the tumour does not increase. Dormancy can be achieved by keeping all tumour cells in cell-cycle arrest (cytostasis) or by balancing the proliferation and death of tumour cells[62]. In the context of heterogeneity, 'genuine dormancy' can be defined as a state in which all tumour cells are in cell-cycle arrest, so that no increase in heterogeneity is possible. 'Pseudo-dormancy' occurs when the numbers of replicating and dying cells are balanced, but as cells are actively proliferating, genetic heterogeneity might still increase in this state. When the number of tumour cells being killed by chemotherapy and the proliferation of resistant cells is balanced, this is only a transient equilibrium, which is a steady state without growth. It can be distinguished from genuine dormancy because it is a process of relapse. In fact, Holmgren and colleagues studied mice that were injected with Lewis lung carcinoma and analysed for metastatic growth, and found that micrometastases became dormant because of the inhibition of angiogenesis and had a threefold higher incidence of apoptosis than cells in growing tumours[63,64]. They reasoned that, in this case, dormancy is attained by a balance between proliferation and apoptosis, which is controlled by angiogenic activity in the tumour microenvironment. The question arises as to what the state of tumour cells is in naturally established dormancy and what mechanisms are involved in this — as opposed to in artificially induced dormancy — particularly in patients who survive for unusually long periods.

Several molecular targets have been manipulated in an attempt to induce dormancy, but none of these strategies — including the downregulation of urokinase-type plasminogen-activator receptor (uPAR)[65–67], and anti-angiogenic and immunotherapeutic strategies[68,69] — have yet yielded outstanding clinical success[62]. For example, angiogenesis inhibitors such as angiotensin and endostatin, which are used to induce dormancy and regression, also circumvent the development of resistance in mice[70,71], but clinical trials of these drugs have not been as successful as had been suggested by the mouse studies[72,73]. In addition, there are reports that primary tumours promote dormancy of micrometastatic tumours in animal models[74–76], as well as a possibly relevant clinical report[77]. At present, a unifying explanation for these diverse observations has not emerged. Taken together, however, the examples cited above indicate that the distinction between

genuine dormancy and pseudo-dormancy is important, and that the induction of genuine dormancy should be a major focus of drug design.

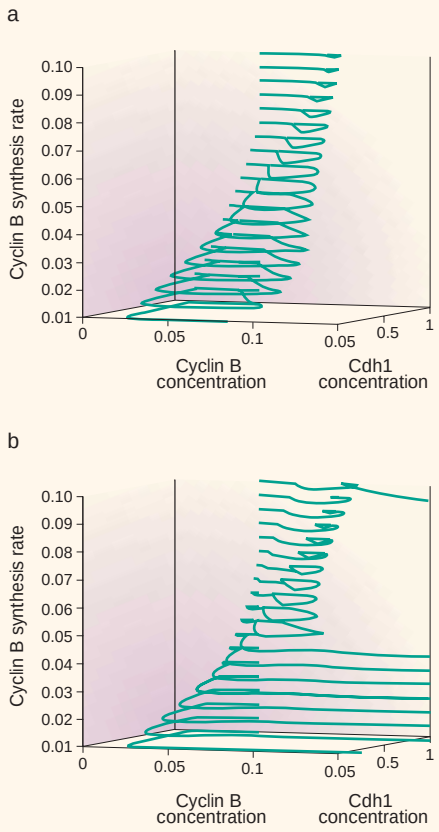

Figure 2 | **Simple computer simulations of robust and fragile cell cycles.** An illustration of how the robustness of the cell cycle can be decreased is shown, using a simplified mathematical model of the budding yeast cell cycle, which reproduces the essential dynamics of the process[127]. Synthesis rates and concentrations are given as arbitrary units. **a** | Trajectories of the concentrations of cyclin B and Cdh1 (Cdc20 homologue 1) using a set of parameters that correspond to wild-type cells when the cyclin B synthesis rate is varied tenfold. The assumed synthesis rate of cyclin B for this model is 0.04 arbitrary units. Despite changes in amplitude and period of oscillation, an oscillatory behaviour of the system, which is shown by the levels of two antagonistic components, Cyclin B and Cdh1, is maintained. **b** | Trajectories produced when one of the kinetic constants is modulated. In this case, the kinetic constant that determines the activation of the Cdc20–APC complex is reduced to 30% of that of the wild-type complex. Oscillatory behaviour is seen only within a limited range of cyclin-synthesis rates (0.05–0.09 arbitrary units). Given that the assumed wild-type rate of cyclin synthesis was 0.04, this modulation enables the termination of oscillatory behaviour by changing the rate of cyclin B synthesis by just 25%, although it is normally robust over a tenfold range.

Controlling cellular dynamics

A major challenge, then, is to control tumours by inducing dormancy or apoptosis without triggering further mutations. This challenge is daunting, specifically because cells are inherently robust and have evolved to use intensive feedback controls to maintain functions, such as progression through the cell cycle and drug resistance, when they are perturbed. Obviously, in addition, therapies must not seriously affect non-tumour cells.

As already mentioned, increased P-gp activity due to the overexpression of *MDR1* is one example of an emergent feedback loop that makes a cell robust against multiple drugs. Inhibitors of P-gp activity, such as verapamil[78], and cyclosporin and its derivative PSC833 (REF. 78), have been used to prevent cells from acquiring multidrug resistance that arises from *MDR1* upregulation[79–81] — this is known as biochemical-modulation chemotherapy[82]. Initial results of clinical trials of this type of therapy have been largely disappointing[83,84]. Although this can be viewed as an early attempt at controlling robustness by eliminating feedback loops or undesirable interactions that counteract the effects of drugs, current biochemical-modulation chemotherapy does not target the complex dynamics of the cell, such as the dynamics of the cell cycle and apoptosis, which is crucial for controlling robust behaviours.

Robustness can only be controlled with a good understanding and thorough analysis of system dynamics. In general, insensitivity to variations in enzyme kinetics and concentrations of chemicals is a network property, rather than a property of a single molecule. However, highly robust circuits that are orchestrated by multilayered feedback controls may be highly vulnerable to failure when a feedback loop is removed. Using a simple model of the cell cycle that incorporates the essential aspects of this process in *Xenopus*, Morohashi *et al.* reported that robustness against changes in various kinetic constants, such as the rate of cyclin synthesis and the rate constants of specific protein–protein interactions, is significantly altered by the modification of regulatory feedback loops[9]. This model showed robust cell-cycle behaviour in response to perturbations, such as changes in several kinetic constants, but if certain feedback loops were removed, the cell cycle became fragile and was easily disrupted by minor perturbations. Similar effects can be achieved by modulating carefully selected feedback loops, without totally removing the feedback (FIG. 2). These computational studies only provide preliminary theoretical results that have not yet been verified by detailed models or by laboratory experiments.

Nevertheless, this work suggests an exciting avenue for research: it might be possible to identify a set of drugs and precise methods of administration — such as order of use, timing and dosage — that would allow an abnormal cellular state to be driven into a desired state, with minimum side-effects. To accomplish this, an adequate computational model of cellular dynamics (for various tumour cell types and normal cells) and a method to systematically explore possible orders of use and choice of chemicals has to be developed, with sufficient experimental verification. The idea of using artificial genetic circuits to actively control the expression of p53 and other tumour suppressors[85] is heading in a similar direction, and can be coordinated within the same framework.

The examples given above provide a conceptual framework for the modulation of robust tumour networks. In reality, however, the complexity of cellular dynamics is underappreciated and, in fact, the perturbation of tumours based on incomplete models of cellular dynamics is likely to unmask unrecognized feedback controls, with potentially damaging results. Nonetheless, research in various areas of cancer biology points to the crucial importance of cellular dynamics in tumour therapy, and indicates that drugs that are targeted to a single molecule are unlikely to be magic bullets.

Several cellular processes have been recognized for which the dynamics involved could be exploited to control robustness. A simple case of temporal perturbations that may shift cellular dynamics has been reported recently. Experiments involving the temporary inactivation of *MYC* have shown that the brief inactivation of a specific gene can affect cellular dynamics, as the subsequent reactivation of *MYC* does not restore the malignant phenotype[86]. These results are contradictory to earlier findings on the effects of *MYC* activation. When tumour cells depend on specific regulatory circuits for their survival and proliferation, the perturbation of such circuits might have a large impact on cellular behaviours and on the underlying dynamics of molecular interactions[87,88].

Recent reports on 'super p53' mice may show that the dynamics of protein networks has a crucial role in tumour robustness. Transgenic mice that constitutively overexpress p53 are resistant to tumorigenesis, but suffer from early ageing and other syndromes. However, super p53 mice, which have an extra copy of *Trp*53 that is under normal regulatory control, are not only resistant to tumorigenesis, but are also free

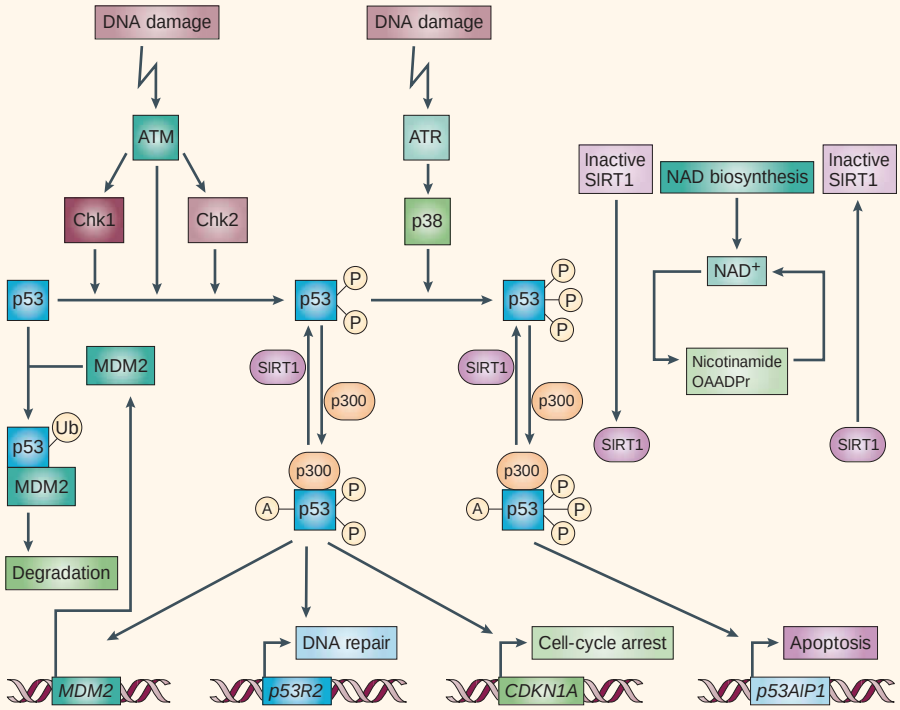

Figure 3 | **Complex interactions involving the p53 tumour suppressor.** DNA damage triggers the phosphorylation of specific residues of p53 by activated kinases (ataxia telangiectasia mutated (ATM), ATM- and RAD3-related (ATR), CHK1, CHK2 and p38). This p53 activation leads to cell-cycle arrest through transcriptional activation (for example, of *CDKN1A*, which encodes WAF1), DNA repair through p53R2 (REF. 93) and other related genes, or apoptosis through genes including p53 apoptosis-inducing protein 1 (*p53AIP1*). Above a certain level of DNA damage, cell-cycle arrest and DNA repair take place — cell-cycle arrest is released when repair is complete. There is also a negative-feedback loop for p53 activity, which involves MDM2. p53 is a transcription factor for the *MDM2* gene, and when MDM2 and p53 form a heterodimer, ubiquitylation and degradation of p53 occurs. SIRT1 (a human homologue of yeast Sir2)[94,95] deacetylates p53, which significantly decreases its DNA-binding ability. The activity of SIRT1 is mainly controlled by NAD homeostasis, which might be related to calorie restriction[128]. p53 activation and deactivation is therefore under complex feedback control and shows various dynamics, including oscillatory and non-oscillatory behaviours and responses to a variety of environmental stimuli. A, acetyl group; OAADPr, *O*-acetyl-ADP-ribose; P, phosphate; Ub, ubiquitin.

from early ageing and other syndromes[89]. When it is under normal regulation, the p53–MDM2 feedback loop causes transient oscillatory behaviours after DNA damage, instead of a constant level of transcription[46]. If such a dynamic behaviour is the crucial reason for the difference between mice with constitutively active p53 and super p53 mice, it is essential that the dynamics of transcription and protein activities are monitored and analysed to inform the development of therapeutic agents.

In fact, p53 is under the regulation of complex multiple-feedback loops[90–92] (FIG. 3), which minimizes the amount of DNA damage that is inherited by dividing cells[93]. In addition, the p53–MDM2 negative-feedback loop that has already been mentioned stabilizes p53 levels, as well as causing oscillatory behaviors of p53 after DNA damage. In addition, p53 activity is also regulated by a homeostatic feedback loop that consists of NAD biosynthesis and the associated activity of SIRT1 (a human homologue of yeast Sir2; REFS 94–99).

Differences in patient outcome can be dependent on the timing of cell-cycle-regulator therapy, and this also illustrates the need to understand cellular dynamics. Outcomes can differ significantly when drugs are given in a different order. For example, if cisplatin is used as a first-line therapy, cells will be arrested in G2, and this drug also interferes with tubulin-polymer formation, making the cells resistant to paclitaxel as a second-line therapy. However, if paclitaxel is given before cisplatin, the effects of cisplatin are augmented, because the uptake of cisplatin is increased in tumour cells and the repair of cisplatin-induced DNA damage is impaired[100]. Cancer chronotherapy — the timed use of chemotherapy, based on circadian rhythms — acknowledges the importance of understanding cellular dynamics to optimize therapy using minimum doses[101–103], as well as exploiting the differences between the cellular rhythms of tumour cells and normal cells[104]. Although it is not yet a part of normal clinical practice, a future direction for the modelling of cellular dynamics, including physiological parameters (such as drug absorption, distribution, metabolism and elimination), may be the identification of specific, optimal, temporal windows for drug therapy.

Systems-based drug discovery

Devising a strategy to explore the innumerable possible combinations of drugs, doses and schedules is an astronomical challenge. New, sound theoretical work and massive computational power are required, combined with high-throughput and high-precision quantitative measurements. First, reference models of both normal cells and tumour cells must be developed, from which an adequate reproduction of dynamic behaviours in response to various stimuli can be analysed. Initially, these models will need to be accurate only in that they reproduce important behaviours at the qualitative level, to allow the comparison of the framework of the model with known experimental data. Well-developed mathematical tools, such as bifurcation analysis, which identifies points of quantitative change in system dynamics, can be adapted to analyse the dynamics of biological systems. This approach has already advanced the understanding of cell-cycle dynamics[105]. A novel mathematical method might provide us with an efficient means of identifying a set of kinetic parameters that makes a system stable or unstable directly from a set of parameter-free equations[106,107]. As the effect of a therapy must be reflected at the cellular level in the form of a change in qualitative behaviours, such as induction of apoptosis and cell-cycle arrest, therapeutic perturbations need to cross the boundaries between qualitatively different dynamic behaviours (FIG. 4).

Tuning the model to be quantitatively consistent with experimental observations requires an extensive effort to measure and estimate critical parameters for *in vivo* molecular interactions. A set of quantitative, and often low-throughput, measurements needs to be used to comprehensively identify the localizations, amounts, interactions and modifications for large numbers of molecular species in timecourse experiments. Although some mathematical methods might help us to reduce the number of parameters that need to be determined experimentally, as well as

suggesting parameters that need to be measured with precision, extensive measurements are likely to be required for the development of a quantitatively accurate model of the cell.

Given this qualitatively and quantitatively accurate model, there is a need to identify a set of perturbations that induces desired changes in cellular behaviours. A large number of combinations for both the selection of perturbations and treatment schedules are anticipated. Further criteria, such as drug availability, toxicity and ADME (absorption, distribution, metabolism and excretion) profiles need to be added to select the final candidate, followed by biological experiments for verification.

Robustness/fragility trade-offs

Theoretical studies of robust systems provide an intriguing possibility for the effective control of targets. Carlson and Doyle have proposed a theory called 'highly optimized tolerance' (HOT), which postulates that systems that acquire robustness against conventional perturbations tend to be extremely fragile to some unexpected perturbations[108–110]. Carlson and Doyle argue that this principle applies to both biological and artificial complex systems. In addition, Csete and Doyle have pointed out a theoretical result that is well-known in control theory, in which the robustness of a system is conserved so that the system being more robust in some aspect is essentially paid for by increased fragility elsewhere. This result, which is similar to the law of the conservation of energy, might also hold for biological systems[110]. This implies that tumour cells that are robust against a wide range of chemical agents may be extremely fragile against certain perturbations. The challenge is to identify the locus of fragility, or to find a method to systematically induce such fragility. Another issue is whether such fragility emerges as a universal trait of variation in tumour cells, or whether each tumour cell has a different type of fragility, so that using a single target, or a limited number of targets, would not eliminate all tumour cells. Even if we identified fragile targets, the diversity of mutations in tumour cells, which generates enormous redundancy, might seriously undermine the efficacy of this approach.

Recently, a hypothesis has been proposed about the existence of error thresholds, at which mutations are accumulated beyond the viability limits of tumour cells[111,112], a situation that is analogous to error-catas-trophe theory for antiviral strategies[113]. Although the common feature

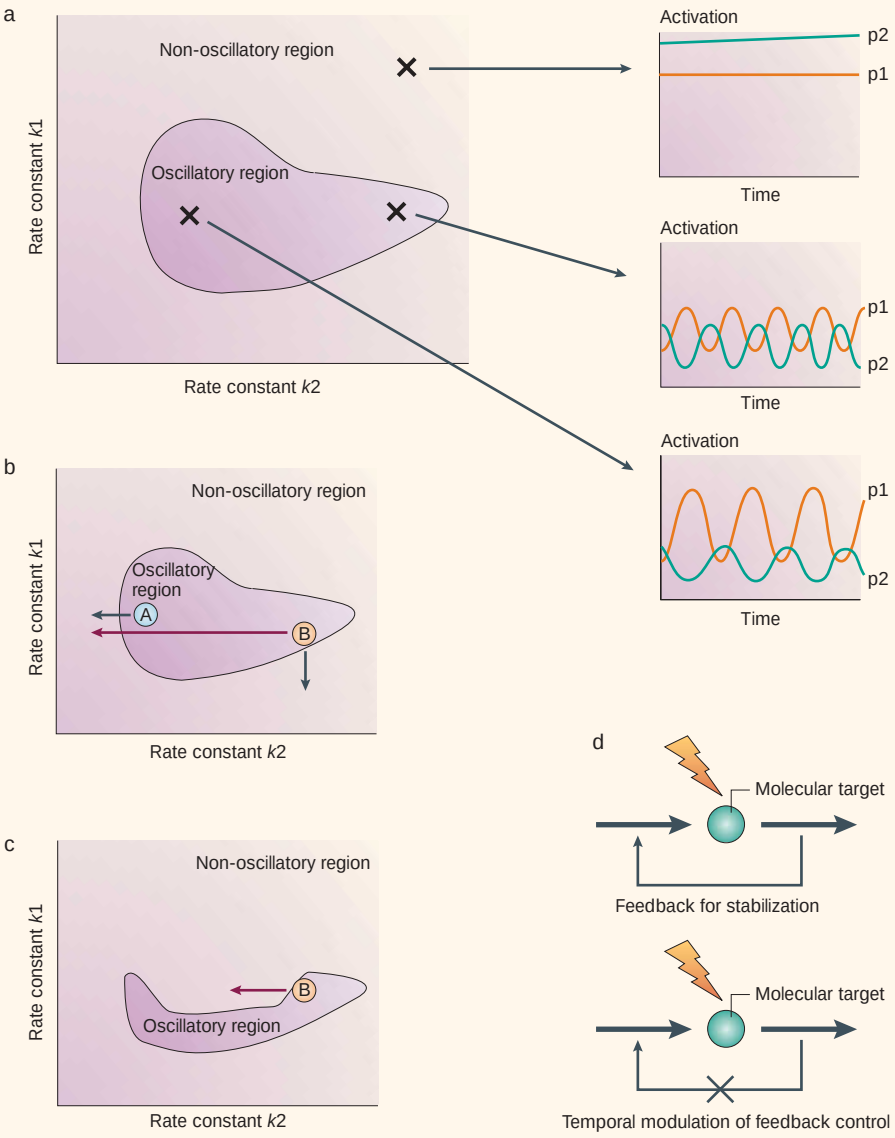

Figure 4 | **Identification and manipulation of phase–space states in cellular dynamics.** In general, behaviours of complex systems can be classified into distinct categories. Systems-based drug discovery explores the dynamics of biological systems in a systematic manner to identify a set of drugs and therapy strategies, rather than targeting a single molecule. The goal of systems-based drug discovery is the identification of the best means of correcting unfavourable dynamics of a system. **a** | Hypothetical example of a phase–space diagram. With different combinations of rate constants (k_1 and k_2), systems behave differently. Changing the rate constants can switch the behaviour of a system — for example, from an oscillatory state, such as cell cycling, to a non-oscillatory state, such as cell-cycle arrest. Within the same region, the system behaves in a qualitatively similar way, but can be quantitatively different. For example, different combinations of two rate constants within the oscillatory region both result in oscillation, but with a different amplitude and frequency. p1, protein 1; p2, protein 2. **b** | Depending on the specific combination of parameters for a cell, different levels of perturbation may be required to switch it into a different state. When the combination of k_1 and k_2 is at the point shown as 'A', a small perturbation that reduces k_2 would stop oscillatory behaviours, such as the cell cycle. However, a larger perturbation is required to stop the oscillation if the combination of k_1 and k_2 is at the point shown as 'B'. In this case, the oscillation of the cell at point B could be easily stopped by reducing k_1 instead. **c** | Alternatively, parameters other than k_1 and k_2 can be manipulated to change the oscillatory region, so that oscillation can be stopped by a small perturbation of k_2. A computational example of such an approach, using a simplified model, is shown in FIG. 2, where perturbation of the kinetic constants for the Cdc20–APC interaction changes the phase space so that the cell cycle can be arrested by a smaller perturbation of the rate of cyclin B synthesis than in the wild-type situation. **d** | Because of feedback loops, there might be cases in which the effects of a drug for a specific molecular target are neutralized. However, if such feedback mechanisms can be inactivated or reduced, at least temporarily, the effect on the targeted molecule will not be neutralized.

of tumour cells is their genetic instability, which is a source of genetic heterogeneity, the proponents of this hypothesis argue that if tumour cells are exposed to further instability, they might cross the error threshold and would not be able to maintain their viability[114]. However, inducing further instability would require the use of a mutagen that might aggravate the situation by affecting both normal and tumour cells. At the same time, tumour cells might respond heterogeneously to such a mutagen, so that not all cells would cross the error threshold, and proliferation by surviving tumour cells would continue. Nevertheless, the idea of viewing genetic instability as a point of fragility is potentially interesting.

Key mechanisms that are involved in tumour responses are crucial for controlling robustness. In this regard, hypoxia-inducible-factor 1 (HIF1) is a master regulator of tumour-cell responses to oxygen, including angiogenesis and metastasis[115], and its function is in some ways analogous to the central role of p53 in regulating responses to DNA damage. This indicates that some aspect of HIF1 regulation might be a point of fragility for tumours. As the dynamics of HIF1 regulation has been well-studied, further elaboration of the differences in HIF1-mediated regulatory cascades in tumours compared with normal cells might point to feedback points of fragility that are unique to tumours. As targeting a single gene tends to have a limited impact due to intratumoral heterogeneity, such a strategy that targeted HIF1 only might generate HIF1-independent responses. So, the real question that needs to be addressed is whether the dynamics of the hypoxia-induced response, which involve HIF1, might confer any fragility that is unique to tumour cells.

Another strategy might be to identify chemicals to which to tumour cells are not normally exposed, as tumour cells that are robust against a range of chemicals might show fragility to such chemicals. However, it is unlikely that all tumour cells simultaneously show fragility to one specific chemical. Again, what needs to be targeted is the fragility of the dynamics of the system, or the basic mechanisms that generates robustness, instead of specific genes and chemicals.

There might be certain conditions that must be met to effectively exploit the fragility that is assumed in the HOT theory. Finding such fragilities requires an in-depth understanding of the dynamics of the gene-regulatory and biochemical networks of tumour cells, as well as genetic instability. Unfortunately, what might constitute a genuine Achilles' heel for cancer is an open question, and major efforts are needed to answer it.

Directions for future research

Considering cancer as a robust system provides us with a framework within which to form strategies for future research. We consider that robustness is attained by redundancy through the genetic heterogeneity of tumour cells and through feedback loops that involve intracellular and host–tumour interactions, so these two mechanisms should be the main targets of cancer therapy.

Although heterogeneity is a well-recognized characteristic of tumours that might lead to drug resistance, the current body of experimental and clinical data that relate to dynamic changes in intratumoral heterogeneity during progression, as well as to responses to various therapeutic strategies, is insufficient. Such studies are important for understanding the basic properties of tumour cells and for providing information that can be used to make decisions about therapeutic strategies. There should also be greater emphasis on developing methods to reduce, or control, the heterogeneity of tumour cells. The recent development of drugs that are targeted against specific molecules, such as imatinib and gefitinib (Iressa), enable the investigation of the precise behaviours of tumour cells in responding to and evading specific perturbations.

In addition, systematic research on how to control cellular states — particularly the induction of dormancy and apoptosis — without triggering mutations should be considered as a crucial component of cancer-therapy research. This requires comprehensive research to understand cellular dynamics with reasonable and practical precision, using computational tools to help to discover a series of perturbations that induce desired cellular states based on available, or technically feasible, sets of drugs, with adequate verification using biological experiments.

The understanding and possible control of complex tumour–host interactions are major challenges that need be tackled. As remodelled tumour microenvironments contain multiple feedback loops to maintain the tumour-promoting properties of the surroundings, effective control points need to be identified to effectively control and revert undesired feedback loops.

Finally, fundamental theoretical investigations into the nature of robustness in biological systems, with specific verification in actual biological systems, needs to be promoted. The HOT theory, for example, provides us with possibilities for new tumour therapies in which fragility could be intentionally induced. Although it is well-known that cancer is robust against a range of therapies, the introduction of this concept, with systematic analysis, provides a series of insights and directions for research. The vast accumulation of experimental and clinical reports can be reinterpreted and reorganized within the framework of how cancer enhances robustness and how to control robustness in clinical practice. Although further investigations are clearly needed to thoroughly examine the validity of this concept, the control of robustness might be an effective guideline for research into cancer therapy, drug discovery and clinical decision-making.

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1. McAdams, H. H. & Arkin, A. Stochastic mechanisms in gene expression. *Proc. Natl Acad. Sci. USA* **94**, 814–819 (1997).
2. McAdams, H. H. & Arkin, A. It's a noisy business! Genetic regulation at the nanomolar scale. *Trends Genet.* **15**, 65–69 (1999).
3. Rao, C. V., Wolf, D. M. & Arkin, A. P. Control, exploitation and tolerance of intracellular noise. *Nature* **420**, 231–237 (2002).
4. Kitano, H. Systems biology: a brief overview. *Science*, **295**, 1662–1664 (2002).
5. Kitano, H. Computational systems biology. *Nature* **420**, 206–210 (2002).
6. Alon, U. *et al.* Robustness in bacterial chemotaxis. *Nature* **397**, 168–171 (1999).
7. Barkai, N. & Leibler, S. Robustness in simple biochemical networks. *Nature* **387**, 913–917 (1997).
8. Yi, T. M. *et al.* Robust perfect adaptation in bacterial chemotaxis through integral feedback control. *Proc. Natl Acad. Sci. USA* **97**, 4649–4653 (2000).
9. Morohashi, M. *et al.* Robustness as a measure of plausibility in models of biochemical networks. *J. Theor. Biol.* **216**, 19–30 (2002).
10. Ueda, H. R., Hagiwara, M. & Kitano, H. Robust oscillations within the interlocked feedback model of *Drosophila* circadian rhythm. *J. Theor. Biol.* **210**, 401–406 (2001).
11. Barkai, N. & Leibler, S. Circadian clocks limited by noise. *Nature* **403**, 267–268 (2000).
12. von Dassow, G. *et al.* The segment polarity network is a robust developmental module. *Nature* **406**, 188–192 (2000).
13. Meir, E. *et al.* Robustness, flexibility, and the role of lateral inhibition in the neurogenic network. *Curr. Biol.* **12**, 778–786 (2002).
14. McAdams, H. H. & Arkin, A. Simulation of prokaryotic genetic circuits. *Annu. Rev. Biophys. Biomol. Struct.* **27**, 199–224 (1998).
15. Little, J. W., Shepley, D. P. & Wert, D. W. Robustness of a gene regulatory circuit. *EMBO J.* **18**, 4299–4307 (1999).
16. Jeong, H. *et al.* The large-scale organization of metabolic networks. *Nature* **407**, 651–654 (2000).
17. Albert, R., Jeong, H. & Barabasi, A. L. Error and attack tolerance of complex networks. *Nature* **406**, 378–382 (2000).
18. Kitano, H. Cancer robustness: tumour tactics. *Nature* **426**, 125 (2003).
19. Harris, J. F. *et al.* Dynamic heterogeneity: characterization of two cell lines derived from experimental lung metastases of mouse KHT fibrosarcoma. *Invasion Metastasis* **7**, 217–229 (1987).
20. Hill, R. P. *et al.* Dynamic heterogeneity: rapid generation of metastatic variants in mouse B16 melanoma cells. *Science* **224**, 998–1001 (1984).

21. Ling, V. *et al.* Dynamic heterogeneity and metastasis. *J. Cell. Physiol.* **3** (Suppl.), 99–103 (1984).
22. Harris, J. F. *et al.* Metastatic variants are generated spontaneously at a high rate in mouse KHT tumor. *Proc. Natl Acad. Sci. USA* **79**, 5547–5551 (1982).
23. Nowell, P. C. Mechanisms of tumor progression. *Cancer Res.* **46**, 2203–2207 (1986).
24. Nowell, P. C. Tumor progression: a brief historical perspective. *Semin. Cancer Biol.* **12**, 261–266 (2002).
25. Gorunova, L. *et al.* Cytogenetic analysis of pancreatic carcinomas: intratumor heterogeneity and nonrandom pattern of chromosome aberrations. *Genes Chromosomes Cancer* **23**, 81–99 (1998).
26. Gorunova, L. *et al.* Extensive cytogenetic heterogeneity in a benign retroperitoneal schwannoma. *Cancer Genet. Cytogenet.* **127**, 148–154 (2001).
27. Fujii, H. *et al.* Frequent genetic heterogeneity in the clonal evolution of gynecological carcinosarcoma and its influence on phenotypic diversity. *Cancer Res*. **60**, 114–120 (2000).
28. Frigyesi, A. *et al.* Power law distribution of chromosome aberrations in cancer. *Cancer Res.* **63**, 7094–7097 (2003).
29. Gonzalez-Garcia, I., Sole, R. V. & Costa, J. Metapopulation dynamics and spatial heterogeneity in cancer. *Proc. Natl Acad. Sci. USA* **99**, 13085–13089 (2002).
30. Baisse, B. *et al.* Intratumor genetic heterogeneity in advanced human colorectal adenocarcinoma. *Int. J. Cancer* **93**, 346–352 (2001).
31. Fearon, E. R. & Vogelstein, B. A genetic model for colorectal tumorigenesis. *Cell* **61**, 759–767 (1990).
32. Lengauer, C., Kinzler, K. W. & Vogelstein, B. Genetic instabilities in human cancers. *Nature* **396**, 643–649 (1998).
33. Tischfield, J. A. & Shao, C. Somatic recombination redux. *Nature Genet.* **33**, 5–6 (2003).
34. Haigis, K. M. & Dove, W. F. A Robertsonian translocation suppresses a somatic recombination pathway to loss of heterozygosity. *Nature Genet.* **33**, 33–39 (2003).
35. Rasnick, D. Aneuploidy theory explains tumor formation, the absence of immune surveillance, and the failure of chemotherapy. *Cancer Genet. Cytogenet.* **136**, 66–72 (2002).
36. Duesberg, P. & Rasnick, D. Aneuploidy, the somatic mutation that makes cancer a species of its own. *Cell Motil. Cytoskeleton* **47**, 81–107 (2000).
37. Li, R. *et al.* Aneuploidy vs. gene mutation hypothesis of cancer: recent study claims mutation but is found to support aneuploidy. *Proc. Natl Acad. Sci. USA* **97**, 3236–3241 (2000).
38. Goldie, J. H. & Coldman, A. J. A mathematic model for relating the drug sensitivity of tumors to their spontaneous mutation rate. *Cancer Treat. Rep.* **63**, 1727–1733 (1979).
39. Skipper, H. E. & Schabel, F. M. Tumor stem cell heterogeneity: implications with respect to classification of cancers by chemotherapeutic effect. *Cancer Treat. Rep.* **68**, 43–61 (1984).
40. Reya, T. *et al.* Stem cells, cancer, and cancer stem cells. *Nature* **414**, 105–111 (2001).
41. Eigen, M. Viral quasispecies. *Sci. Am.* **269**, 42–49 (1993).
42. Juliano, R. L. & Ling, V. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. *Biochim. Biophys. Acta* **455**, 152–162 (1976).
43. Nooter, K. & Herweijer, H. Multidrug resistance (*mdr*) genes in human cancer. *Br. J. Cancer* **63**, 663–669 (1991).
44. Zhang, R. & Wang, H. *MDM2* oncogene as a novel target for human cancer therapy. *Curr. Pharm. Des.* **6**, 393–416 (2000).
45. Zheleva, D. I., Lane, D. P. & Fischer, P. M. The p53–Mdm2 pathway: targets for the development of new anticancer therapeutics. *Mini Rev. Med. Chem.* **3**, 257–270 (2003).
46. Lev Bar-Or, R. *et al.* Generation of oscillations by the p53–Mdm2 feedback loop: a theoretical and experimental study. *Proc. Natl Acad. Sci. USA* **97**, 11250–11255 (2000).
47. Radisky, D., Hagios, C. & Bissell, M. J. Tumors are unique organs defined by abnormal signaling and context. *Semin. Cancer Biol.* **11**, 87–95 (2001).
48. Bissell, M. J. & Radisky, D. Putting tumours in context. *Nature Rev. Cancer* **1**, 46–54 (2001).
49. Kizaka-Kondoh, S. *et al.* Tumor hypoxia: A target for selective cancer therapy. *Cancer Sci.* **94**, 1021–1028 (2003).
50. Hockel, M. *et al.* Association between tumor hypoxia and malignant progression in advanced cancer of the uterine cervix. *Cancer Res.* **56**, 4509–4515 (1996).
51. Hockel, M. & Vaupel, P. Biological consequences of tumor hypoxia. *Semin. Oncol.* **28** (Suppl. 8), 36–41 (2001).
52. Hockel, M. & Vaupel, P. Tumor hypoxia: definitions and current clinical, biologic, and molecular aspects. *J. Natl Cancer Inst.* **93**, 266–276 (2001).
53. Harris, A. L. Hypoxia — a key regulatory factor in tumour growth. *Nature Rev. Cancer* **2**, 38–47 (2002).
54. Goonewardene, T. I., Sowter, H. M. & Harris, A. L. Hypoxia-induced pathways in breast cancer. *Micros. Res. Tech.* **59**, 41–48 (2002).
55. Goldie, J. H. & Coldman, A. J. The genetic origin of drug resistance in neoplasms: implications for systemic therapy. *Cancer Res*. **44**, 3643–3653 (1984).
56. Boccardo, F. *et al.* Lack of effectiveness of adjuvant alternating chemotherapy in node-positive, estrogen-receptor-negative premenopausal breast cancer patients: results of a multicentric Italian study. The Breast Cancer Adjuvant Chemo-Hormone Therapy Cooperative Group (GROCTA). *Cancer Invest*. **15**, 505–512 (1997).
57. Chacon, R. *et al.* Less efficacy with alternating regimen as adjuvant chemotherapy in stage II node-positive breast cancer: results at 8 years (Pronacam 85). *Br. J. Cancer* **76**, 545–550 (1997).
58. Hochhaus, A. Cytogenetic and molecular mechanisms of resistance to imatinib. *Semin. Hematol.* **40** (Suppl. 3), 69–79 (2003).
59. Gorre, M. E. *et al.* Clinical resistance to STI-571 cancer therapy caused by *BCR–ABL* gene mutation or amplification. *Science* **293**, 876–880 (2001).
60. Hochhaus, A. *et al.* Roots of clinical resistance to STI–571 cancer therapy. *Science* **293**, 2163 (2001).
61. Barthe, C. *et al*. Roots of clinical resistance to STI–571 cancer therapy. *Science* **293**, 2163 (2001).
62. Uhr, J. W. *et al.* Cancer dormancy: opportunities for new therapeutic approaches. *Nature Med*. **3**, 505–509 (1997).
63. Holmgren, L., O'Reilly, M. S. & Folkman, J. Dormancy of micrometastases: balanced proliferation and apoptosis in the presence of angiogenesis suppression. *Nature Med.* **1**, 149–153 (1995).
64. Murray, C. Tumour dormancy: not so sleepy after all. *Nature Med.* **1**, 117–118 (1995).
65. Aguirre Ghiso, J. A., Kovalski, K. & Ossowski, L. Tumor dormancy induced by downregulation of urokinase receptor in human carcinoma involves integrin and MAPK signaling. *J. Cell Biol.* **147**, 89–104 (1999).
66. Aguirre-Ghiso, J. A. *et al*. ERK (MAPK) activity as a determinant of tumor growth and dormancy; regulation by p38 (SAPK). *Cancer Res*. **63**, 1684–1695 (2003).
67. Aguirre-Ghiso, J. A. *et al.* Urokinase receptor and fibronectin regulate the ERK (MAPK) to p38 (MAPK) activity ratios that determine carcinoma cell proliferation or dormancy *in vivo*. *Mol. Biol. Cell* **12**, 863–879 (2001).
68. Schirrmacher, V. T-cell immunity in the induction and maintenance of a tumour dormant state. *Semin. Cancer Biol.* **11**, 285–295 (2001).
69. Schirrmacher, V. *et al.* T cell memory, anergy and immunotherapy in breast cancer. *J. Mammary Gland Biol. Neoplasia* **7**, 201–208 (2002).
70. O'Reilly, M. S. *et al.* Angiostatin induces and sustains dormancy of human primary tumors in mice. *Nature Med.* **2**, 689–692 (1996).
71. Boehm, T. *et al.* Antiangiogenic therapy of experimental cancer does not induce acquired drug resistance. *Nature* **390**, 404–407 (1997).
72. Drevs, J. *et al.* Antiangiogenesis: current clinical data and future perspectives. *Onkologie* **25**, 520–527 (2002).
73. McCarty, M. F. *et al.* Promises and pitfalls of anti-angiogenic therapy in clinical trials. *Trends Mol. Med.* **9**, 53–58 (2003).
74. Naumov, G. N. *et al.* Persistence of solitary mammary carcinoma cells in a secondary site: a possible contributor to dormancy. *Cancer Res* **62**, 2162–2168 (2002).
75. Naumov, G. N. *et al.* Solitary cancer cells as a possible source of tumour dormancy? *Semin. Cancer Biol.* **11**, 271–276 (2001).
76. Guba, M. *et al.* A primary tumor promotes dormancy of solitary tumor cells before inhibiting angiogenesis. *Cancer Res*. **61**, 5575–5579 (2001).
77. De Giorgi, V. *et al.* Immediate local and regional recurrence after the excision of a polypoid melanoma: tumor dormancy or tumor activation? *Dermatol. Surg.* **29**, 664–667 (2003).
78. Tsuruo, T. *et al.* Overcoming of vincristine resistance in P388 leukemia *in vivo* and *in vitro* through enhanced cytotoxicity of vincristine and vinblastine by verapamil. *Cancer Res.* **41**, 1967–1972 (1981).
79. Kaye, S. B. Multidrug resistance: clinical relevance in solid tumours and strategies for circumvention. *Curr. Opin. Oncol.* **10** (Suppl. 1), S15–S19 (1998).
80. Krishna, R. & Mayer, L. D. Multidrug resistance (MDR) in cancer. Mechanisms, reversal using modulators of MDR and the role of MDR modulators in influencing the pharmacokinetics of anticancer drugs. *Eur. J. Pharm. Sci.* **11**, 265–283 (2000).
81. Krishna, R. & Mayer, L. D. Modulation of P-glycoprotein (PGP) mediated multidrug resistance (MDR) using chemosensitizers: recent advances in the design of selective MDR modulators. *Curr. Med. Chem. Anti-Canc. Agents* **1**, 163–174 (2001).
82. Peters, G. J., Schornagel, J. H. & Milano, G. A. Clinical pharmacokinetics of anti-metabolites. *Cancer Surv.* **17**, 123–156 (1993).
83. Rodenburg, C. J. *et al.* Phase II study of combining vinblastine and cyclosporin-A to circumvent multidrug resistance in renal cell cancer. *Ann. Oncol.* **2**, 305–306 (1991).
84. Verweij, J. *et al.* A phase II study of epidoxorubicin in colorectal cancer and the use of cyclosporin-A in an attempt to reverse multidrug resistance. *Br. J. Cancer* **64**, 361–364 (1991).
85. Hasty, J., McMillen, D. & Collins, J. J. Engineered gene circuits. *Nature* **420**, 224–230 (2002).
86. Jain, M. *et al.* Sustained loss of a neoplastic phenotype by brief inactivation of MYC. *Science* **297**, 102–104 (2002).
87. Weinstein, I. B. Addiction to oncogenes — the Achilles' heal of cancer. *Science* **297**, 63–64 (2002).
88. Weinstein, I. B. Disorders in cell circuitry during multistage carcinogenesis: the role of homeostasis. *Carcinogenesis* **21**, 857–864 (2000).
89. Garcia-Cao, I. *et al.* 'Super p53' mice exhibit enhanced DNA damage response, are tumor resistant and age normally. *EMBO J.* **21**, 6225–6235 (2002).
90. Vogelstein, B., Lane, D. & Levine, A. J. Surfing the p53 network. *Nature* **408**, 307–310 (2000).
91. Jin, S. & Levine, A. J. The p53 functional circuit. *J. Cell Sci.* **114**, 4139–4140 (2001).
92. Giaccia, A. J. & Kastan, M. B. The complexity of p53 modulation: emerging patterns from divergent signals. *Genes Dev.* **12**, 2973–2983 (1998).
93. Tanaka, H. *et al.* A ribonucleotide reductase gene involved in a p53-dependent cell-cycle checkpoint for DNA damage. *Nature* **404**, 42–49 (2000).
94. Imai, S. *et al.* Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature* **403**, 795–800 (2000).
95. Luo, J. *et al.* Negative control of p53 by Sir2α promotes cell survival under stress. *Cell* **107**, 137–148 (2001).
96. Smith, J. Human Sir2 and the 'silencing' of p53 activity. *Trends Cell Biol.* **12**, 404–406 (2002).
97. Denu, J. M. Linking chromatin function with metabolic networks: Sir2 family of NAD^+-dependent deacetylases. *Trends Biochem. Sci.* **28**, 41–48 (2003).
98. Guarente, L. Sir2 links chromatin silencing, metabolism, and aging. *Genes Dev.* **14**, 1021–1026 (2000).
99. Koubova, J. & Guarente, L. How does calorie restriction work? *Genes Dev.* **17**, 313–321 (2003).
100. Shah, M. A. & Schwartz, G. K. Cell cycle-mediated drug resistance: an emerging concept in cancer therapy. *Clin. Cancer Res.* **7**, 2168–2181 (2001).
101. Halberg, F. *et al.* Toward a chronotherapy of neoplasia: tolerance of treatment depends upon host rhythms. *Experientia* **29**, 909–934 (1973).
102. Levi, F. Chronobiology and cancer. *Pathol Biol.* (Paris) **35**, 960–968 (1987).
103. Mormont, M. C. & Levi, F. Cancer chronotherapy: principles, applications, and perspectives. *Cancer* **97**, 155–169 (2003).
104. Blagosklonny, M. V. & Pardee, A. B. Exploiting cancer cell cycling for selective protection of normal cells. *Cancer Res*. **61**, 4301–4305 (2001).
105. Borisuk, M. T. & Tyson, J. J. Bifurcation analysis of a model of mitotic control in frog eggs. *J. Theor. Biol.* **195**, 69–85 (1998).
106. Prajna, S. & Papachristodoulou, A. Analysis of switched and hybrid systems — beyond piecewise quadratic methods. *Proc. Am. Control Conf*. (2003).
107. Prajna, S., Papachristodoulou, A. & Parrilo, P. A. Introducing SOSTOOLS: a general purpose sum of square programming solver. *Proc. IEEE Conf. Decision Control* (2002).
108. Carlson, J. M. & Doyle, J. Highly optimized tolerance: a mechanism for power laws in designed systems. *Phys. Rev. E Stat. Phys. Plasmas Fluids Relat. Interdiscip. Topics* **60**, 1412–1427 (1999).
109. Carlson, J. M. & Doyle, J. Complexity and robustness. *Proc. Natl Acad. Sci. USA* **99** (Suppl. 1), 2538–2545 (2002).
110. Csete, M. E. & Doyle, J. C. Reverse engineering of biological complexity. *Science* **295**, 1664–1669 (2002).
111. Sole, R. V. Phase transitions in unstable cancer cell populations. *Eur. Phys. J.* **B**,117–123 (2003).
112. Cahill, D. P. *et al.* Genetic instability and darwinian selection in tumours. *Trends Cell. Biol.* **9**, M57–M60 (1999).
113. Eigen, M. Error catastrophe and antiviral strategy. *Proc. Natl Acad. Sci. USA* **99**, 13374–13376 (2002).
114. Loeb, L. A., Loeb, K. R. & Anderson, J. P. Multiple mutations and cancer. *Proc. Natl Acad. Sci. USA* **100**, 776–781 (2003).

115. Semenza, G. L. Targeting HIF-1 for cancer therapy. *Nature Rev. Cancer* **3**, 721–732 (2003).
116. Levy, A. P., Levy, N. S. & Goldberg, M. A. Hypoxia-inducible protein binding to vascular endothelial growth factor mRNA and its modulation by the von Hippel-Lindau protein. *J. Biol. Chem.* **271**, 25492–25497 (1996).
117. Damert, A., Ikeda, E. & Risau, W. Activator-protein-1 binding potentiates the hypoxia-inducible-factor-1-mediated hypoxia-induced transcriptional activation of vascular-endothelial growth factor expression in C6 glioma cells. *Biochem. J.* **327**, 419–423 (1997).
118. Mazure, N. M. *et al.* Oncogenic transformation and hypoxia synergistically act to modulate vascular endothelial growth factor expression. *Cancer Res.* **56**, 3436–3440 (1996).
119. Staller, P. *et al.* Chemokine receptor CXCR4 downregulated by von Hippel-Lindau tumour suppressor pVHL. *Nature* **425**, 307–311 (2003).
120. Pennacchietti, S. *et al.* Hypoxia promotes invasive growth by transcriptional activation of the *met* protooncogene. *Cancer Cell* **3**, 347–361 (2003).
121. Muller, A. *et al.* Involvement of chemokine receptors in breast cancer metastasis. *Nature* **410**, 50–56 (2001).
122. Mazurek, S. & Eigenbrodt, E.The tumor metabolome. *Anticancer Res.* **23**, 1149–1154 (2003).
123. Kenny, P. A. & Bissell M. J. Tumor reversion: correction of malignant behavior by microenvironmental cues. *Int. J. Cancer* **107**, 688–695 (2003).
124. Homey, B., Muller, A. & Zlotnik, A. Chemokines: agents for the immunotherapy of cancer? *Nature Rev. Immunol.* **2**, 175–184 (2002).
125. Liotta, L. A. & Kohn, E. C. The microenvironment of the tumour–host interface. *Nature* **411**, 375–379 (2001).
126. Parish, C. R. Cancer immunotherapy: the past, the present and the future. *Immunol. Cell Biol.* **81**, 106–113 (2003).
127. Tyson, J. J. & Novak, B. in *Computational Cell Biology* (eds Fall, C., Marland, E., Wagner, J. & Tyson, J.) 261–284 (Springer-Verlag, New York, 2002).
128. Hursting, S. D. *et al.* Calorie restriction, aging, and cancer prevention: mechanisms of action and applicability to humans. *Annu. Rev. Med.* **54**, 131–52 (2003).

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The author declares that he has no competing financial interests.

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SCIENCE AND SOCIETY

Genetic testing for cancer susceptibility: the promise and the pitfalls

Caryn Lerman and Alexandra E. Shields

Genetic testing for hereditary cancer risk is now available and has the potential to reduce cancer mortality through the targeting of preventive therapies and by motivating behavioural change. However, generating and communicating genetic information can have psychological and social consequences. As testing extends from identifying rare hereditary cancers to testing for common genetic variants that are associated with cancer risk, how do we address these complex problems to maximize the benefits of genetic testing?

The number of known genetic mutations that are associated with cancer susceptibility is growing at an exponential rate[1], and the use of genetic testing for cancer susceptibility is becoming more widespread. Genetic testing is now available for the main cancer susceptibility genes, in which rare mutations predispose to uncommon inherited cancer syndromes, such as hereditary breast and ovarian cancer (HBOC), hereditary non-polyposis colon cancer (HNPCC) and familial adenomatous polyposis (FAP). The list of available genetic tests for hereditary cancer syndromes is shown in TABLE 1, and a detailed review of cancer syndromes and laboratories that are performing research and clinical genetic testing can be found at the GeneTests web site (see online links box).

The specific processes and outcomes of genetic testing for cancer susceptibility are shown in FIG. 1, which uses genetic testing for HBOC as an example. As with other forms of genetic testing for disease susceptibility[2], following a detailed family- and personal-history assessment, the genetic counsellor, medical geneticist or other health professional provides an individual with a genetic risk assessment, based on the information collected, and reviews the benefits, limitations and risks of genetic testing[3]. The individual must then decide whether to proceed with testing and the appropriate biological samples are collected and analysed. Once the results are obtained, the individual must provide written informed consent to participate in an in-person counselling visit, during which the results are disclosed and interpreted and options for medical management are addressed.

For hereditary cancer syndromes, such as HBOC, a positive test result for a known disease-conferring mutation indicates that an individual has an increased risk of developing cancer; however, it is by no means certain that such individuals will develop cancer. By contrast, a negative test result in a family with a known deleterious mutation provides definitive information that the individual has only an average or 'general population' risk of developing HBOC. Cancer could still develop in such a person though, as a result of other genetic and/or environmental factors. In families in which the specific disease-conferring mutation has not yet been identified, a positive result for a novel mutation or a negative genetic test result would not be informative. It should be noted, however, that there are important differences in the counselling process, implications and outcomes of genetic testing for the different hereditary cancer syndromes. This review focuses primarily on HBOC and HNPCC as examples.

Recent research has begun to identify common genetic variants that augment the effects of risk-factor exposure, such as genes that affect the metabolism of hormones or that predispose individuals to behaviours that are associated with cancer risk ('cancer-risk behaviours'); for example, genes that predispose an individual to tobacco addiction. However, risk factors/behaviours that are associated with cancer, such as tobacco use and obesity, are traits that are influenced by a complex interplay of numerous genetic, psychosocial and environmental factors. Therefore, future testing for such traits would provide less information than tests that are used for hereditary cancers, as the test results would have a much higher level of uncertainty, even if positive. A comparison of the features of the main cancer-predisposing genes compared with genes that are associated with complex traits is shown in TABLE 2.

Although the full impact of genetics on clinical care is yet to be realized and remains uncertain, it is anticipated that genetic testing for cancer susceptibility could eventually allow physicians to identify individuals who are susceptible to certain types of cancer, and thereby allow them to tailor preventive and