Proliferation, cell cycle and apoptosis in cancer

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Beneath the complexity and idiopathy of every cancer lies a limited number of 'mission critical' events that have propelled the tumour cell and its progeny into uncontrolled expansion and invasion. One of these is deregulated cell proliferation, which, together with the obligate compensatory suppression of apoptosis needed to support it, provides a minimal 'platform' necessary to support further neoplastic progression. Adroit targeting of these critical events should have potent and specific therapeutic consequences.

Since its inception, the study of the molecular basis of cancer has carried with it the promise of more refined, more effective cancer therapies. It has generally been assumed that because cancers are derived from numerous tissues with multiple aetiologies, and as tumour progression carries with it a bewildering and seemingly endless combination of genetic and epigenetic alterations giving rise to a hugely disparate series of diseases, cures for cancer must be as diverse as the diseases themselves. The mantra from the cancer research community has been that cancer is not a single disease for which there will be a single cure, and the task of developing therapies suitable for treatment of the full gamut of cancers is depicted as Herculean and almost impossible.

In this review, we entertain the idea that these assertions are unnecessarily pessimistic. Although cancers are indeed extremely diverse and heterogeneous, we suggest that underlying this variability lies a relatively small number of 'mission critical' events whose convergence is required for the development of any and all cancers. The focus of this perspective is on two of these: the lesions that power the relentless proliferation of tumour cells, and the compensatory mutations that arise to ensure their survival. Although neoplasia involves many other processes that also present targets for cancer therapy[1], in almost all instances, deregulated cell proliferation and suppressed cell death together provide the underlying platform for neoplastic progression. The challenge before the research community is to identify and understand the molecular anatomy of such pivotal steps in tumour progression and to develop therapies that directly attack these points of convergence.

Evolution of cancers

Cancers are diseases in which unremitting clonal expansion of somatic cells kills by invading, subverting and eroding normal tissues. Driving cancer development are stochastic somatic cell mutations in genes that govern and regulate the diverse aspects of metazoan growth control. The processes governing the genesis and progression of cancers are evolutionary ones in which natural selection acts upon the inherent or acquired diversity of various somatic clones, fostering the outgrowth of those with some form of propagative advantage. Metazoans must restrain this tendency of individual somatic cells to establish their own autonomous colonies, yet at the same time sanction sufficient somatic cell proliferation to build and maintain the whole organism. The solution adopted by most animals is simple: adults are small, short-lived and disposable egg dispersers, constructed almost exclusively of post-mitotic cells whose irreversible loss of proliferative capacity effectively curtails any opportunity for mutation and somatic evolution.

Unfortunately, long-lived organisms such as vertebrates need substantial and continuous cell proliferation throughout their extended lives, both for development and long-term maintenance and repair. In teleological terms, the evolutionary imperative of vertebrates has been to find a way to allow cell proliferation when needed, while at the same time efficiently suppressing the genesis of mutated cells leading to deregulated growth. When such measures fail, cancer is the inevitable consequence.

Awareness of the evolutionary nature of cancer offers a number of important insights into the malignant process. First, and perhaps most striking, is the rarity of the cancer cell. With an estimated mutation rate of some 1 in 2×10^7 per gene cell division[2], some 10^{14} target cells in the average human, and an abundant repertoire of genes regulating all aspects of cell expansion, it is remarkable that cancers arise in only 1 in 3 lifetimes. This is even more striking when one considers that oncogenic mutations, by their nature, foster clonal expansion of the affected cell, so propagating the initial mutation and thereby increasing the number of target cells available for (and hence the probability of) further oncogenic mutation. The rarity of cancer highlights the efficacy of potent anti-tumorigenic mechanisms presiding over somatic cells. Cancers prevail only when these mechanisms have failed[3].

Second, cancers 'progress' for the same reason organisms seem to — we see only the successes, not the failures. This distorts our statistical view of cancer progression. No matter how rare the genesis and evolution of a cancer cell or how effective the anti-cancer therapy administered, our perception is only of the rare surviving clones that beat all the odds and appear as clinical disease. Our inability to discern the mechanisms that thwart the vast majority of inchoate tumours deprives us of great insight into how these mechanisms break down in cancer and, correspondingly, how we might best reactivate them.

Third, evolutionary trajectories of cancers are shaped by the selective pressures they encounter. Tumours evolve within differing somatic environments, each of which imposes its own unique constraints. For example, shedding epithelia such as gut or skin 'defend' themselves against the emergence of sizeable mutant clones by condemning all

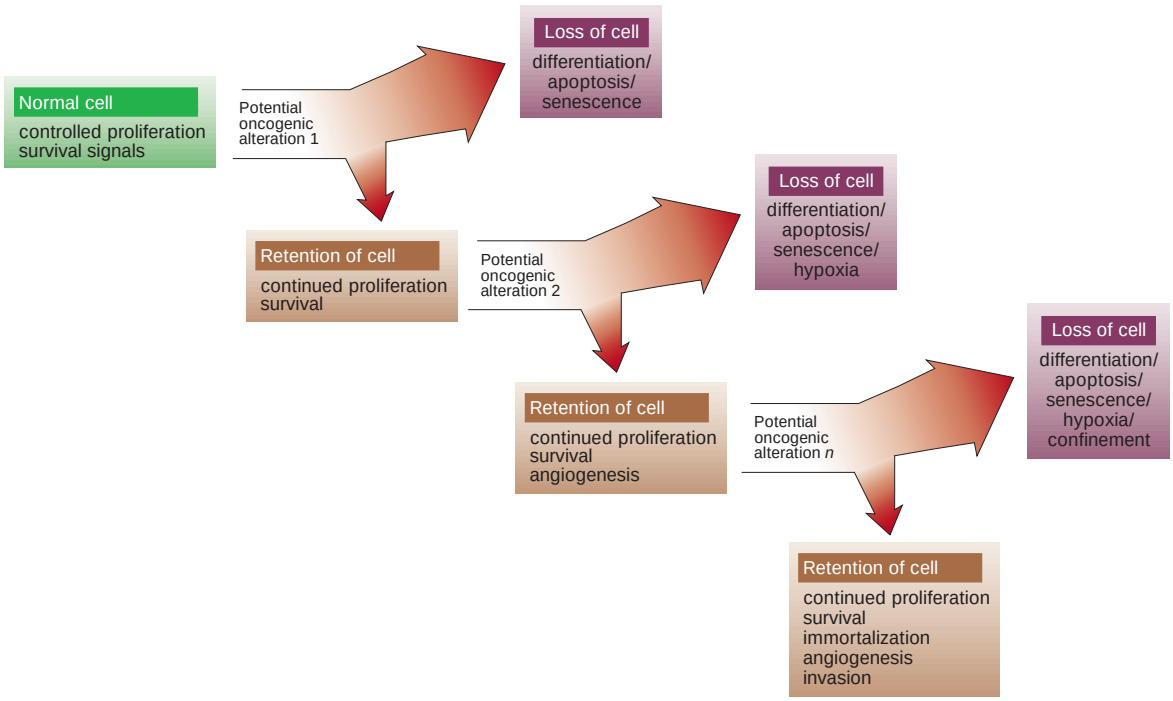

Figure 1 Evolution of cancer is more complex than the straightforward linear accumulation of oncogenic mutations. Potentially oncogenic proliferative signals are coupled to a variety of growth-inhibitory processes, such as the induction of apoptosis, differentiation or senescence, each of which restricts subsequent clonal expansion and neoplastic evolution. Tumour progression occurs only in the very rare instances where these growth-inhibitory mechanisms are thwarted by compensatory mutations.

progeny cells to terminal differentiation and death. Derailing of this differentiating conveyer belt is an important part of gastrointestinal and skin cancer, but is clearly irrelevant to the process of carcinogenesis in a tissue such as liver.

Fourth, evolution is an ongoing process. As a neoplasm progresses, expands and spreads, it confronts shifting selective pressures. The heterogeneity and diversity seen in cancers are vestiges of a dynamic and stochastic evolutionary force that varies with differing somatic environments.

The commonality of cancers

Tumours are diverse and heterogeneous, but all share the ability to proliferate beyond the constraints limiting growth in normal tissue. Aberrations in the regulation of a restricted number of key pathways that control cell proliferation and cell survival are mandatory for establishment of all tumours. Deregulated cell proliferation together with suppressed apoptosis constitute the minimal common platform upon which all neoplastic evolution occurs. The critical issue is to identify how tumour cells differ from normal cells and how those differences can be exploited therapeutically.

Limits to clonal autonomy of metazoan cells

The restriction of clonal autonomy that is essential in vertebrate biology is implemented by tiers of mechanisms, each one of which must be somehow evaded or negated for cancers to arise (Fig. 1).

Normal somatic cells are totally dependent for their proliferation upon receipt of appropriate mitogenic signals. Mitogens act as obligate social cues that constrain cells to proliferate only in the appropriate social context. Furthermore, cells become committed to entry of the cell cycle only towards the end of G1, a retinoblastoma (pRB)-regulated transition point which most cell types reach only after hours or days of sustained mitogen exposure[4]. Thus, cells will respond only to proliferative impetuses of some tenacity. In some cases, sustained mitogenic signalling can only occur within a specific somatic context. For example, the transient and mitogenically inadequate induction of cyclin D1, induced by mitogen activation of receptor tyrosine kinase (RTK) signalling, is transmuted into a persistent and mitogenically productive response upon co-stimulation of integrins via attachment to the extracellular matrix (ECM)[5].

Superimposed upon the requirement for positive growth signals lies a web of growth inhibitory factors that serve to gate the proliferative response to mitogens, and which has to be overcome for cell-cycle entry[1]. Examples of such factors are transforming growth factor-β[6] and the interferons[7]. These pleiotropic signalling molecules exert potent anti-proliferative effects, in part by suppressing phosphorylation of pRB, through their inhibitory effects on cyclin-dependent kinases (CDKs) and induction of various CDK inhibitors, and also by their suppression of c-Myc.

The inverse coupling of differentiation to proliferation is another hardwired restraint to somatic cell autonomy, as proliferative potential of somatic cells is counterbalanced by an innate predisposition of progeny cells to engage pathways of terminal differentiation[8]. Moreover, unfettered proliferative potential is restricted to a small number of slowly replicating stem cells. These typically undergo infrequent asymmetric divisions, generating one daughter that replaces the original, while the other enters a transit amplifying population resulting in irreversible commitment to a terminal differentiation programme. By confining most cell expansion to cells already committed to ultimate genetic or physical death, stem cells allow provision of sufficient cells to maintain and replace tissues, while restricting the number of cell divisions (and hence exposure to mutagenic risk) in those somatic cells with significant proliferative potential[9–11].

Somatic cells that evolve the capacity for proliferative autonomy still face major obstacles to their continued expansion. Metazoan somatic cells are obligatorily dependent for their survival upon the continuous availability of trophic factors, which are often in limiting supply and spatially restricted[12,13]. Consequently, deregulated cell expansion results

in exhaustion of local survival factors and the triggering of apoptosis. Furthermore, many rapidly proliferating epithelial tissues have evolved architectures that ensure the eventual death of progeny cells as they are forced to migrate outwards to be shed from the surface. Should rare clones then succeed in evading both growth control and death, they then encounter the ultimate proliferative backstop. Repeated divisions erode their telomeres, ultimately triggering irreversible arrest or, more likely, apoptosis[14]. Finally, to form a tumour the errant clone must make its way in the outside world of somatic tissues. Substantial evidence indicates that development of macroscopic metastatic cancers requires the capacity to erode and subvert normal tissues and commandeer a nurturing vasculature from pre-existing blood vessels in adjacent normal tissues (see article in this issue by Liotta and Kohn, pages 375–379).

Cancer as a disease of deregulated cell proliferation

Each of the pathways that constrains the proliferative response in normal cells is perturbed in most cancers. One class of mutations required for tumour development acts by short circuiting the normally obligate requirement of somatic cells for external mitogenic signals[15]. Such mutations may involve autocrine production of a normally limiting mitogen, activating mutations of the mitogen RTKs or G-protein signal transducers such as Ras, or mutations affecting one of the many intermediary signal transducing molecules that convey mitogenic information to its intracellular targets (see review in this issue by Blume-Jensen and Hunter, pages 355–365). A second class of growth-deregulating mutations comprises those that target the principal late-G1 cell-cycle checkpoint regulated by pRB[16]. Defects in this pathway, which may be universal in human cancers, include deletion of the *RB* gene itself and deregulation of the CDKs that phosphorylate and functionally inactivate pRB, either through direct over-activation of CDKs or through genetic loss of their inhibitors[17]. Another frequent proliferative lesion that has the effect of deregulating the cell cycle is uncontrolled expression of Myc[18]. Myc expression is tightly controlled by mitogen availability in normal cells, but it is usually expressed in a deregulated or elevated manner in tumour cells. Myc seems to be a strategic controller of cell proliferation that acts pleiotropically to coordinate both cell growth[19–21] and concomitant progression through the cell cycle[22,23].

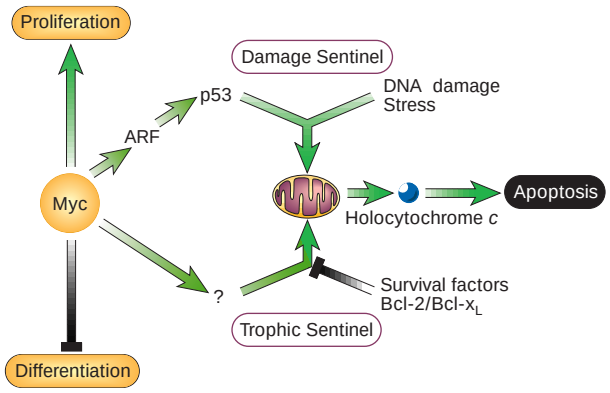

Figure 2 Activation of growth-deregulating lesions triggers 'sentinel' functions that guard the cell against acquiring mutations or propagating into an inappropriate somatic compartment. The more powerful and persistent the growth signal, the more potent and persistent the sentinel function. In this example, the oncoprotein Myc is shown activating a p53 damage sentinel through the ARF/MDM-2 pathway, thereby sensitizing the cell to any DNA damage. Myc also promotes release of holocytochrome *c* from the mitochondrion into the cytosol where it triggers apoptosis. Release of holocytochrome *c* is inhibited by paracrine 'survival' signals that are typically restricted both in supply and location. Clonal outgrowth driven by relentless Myc expression outstrips survival factor availability, triggering the 'trophic sentinel' to kill the cell.

The presence in individual tumours of multiple mutations that affect each of the pathways discussed above suggests that each pathway contributes a discrete type of proliferative function to the neoplastic phenotype. But precisely what such functions are and how and why they interact, remains unknown. Moreover, in certain circumstances single types of proliferative lesion seem sufficient to drive cell proliferation. For example, mere deregulation of c-Myc is, at least in the mouse, alone sufficient to induce and maintain proliferation of multiple somatic cell types *in vitro* and *in vivo*[24,25].

In addition to driving aberrant cell division, mutations in the various proliferative control pathways have a profound impact on other cell functions. For example, many of the proliferative lesions in tumour cells also contribute to the inhibition of differentiation, thereby preventing the elimination of progeny cells from the proliferative compartment of many types of tissue. pRB, for example, is essential in differentiation of several tissue types through interactions with factors such as the helix–loop–helix proteins MyoD[26] and Id2 (ref. 27). Loss or inhibition of pRB function prevents normal differentiation, a contribution to tumour development distinct from the direct deregulation of cell-cycle progression. Deregulated Myc expression also inhibits differentiation, in part by activation of Id2 expression[27].

Cancer as a disease of deregulated survival

Survival of all somatic cells requires the continuous input of survival and trophic signals to suppress apoptosis. The central engines of apoptosis are the caspases, cascades of cysteine aspartyl proteases that implement cell death by cleaving a variety of intracellular substrates that trigger cell dissolution. Caspases are synthesized as latent zymogens that are activated by proteolytic cleavage: typically through the action of upstream apical caspases. One such pathway is mediated by transmembrane death receptors of the CD95 (Apo-1 or Fas)/TRAIL/tumour-necrosis factor (TNF) receptor 1 family, whose ligation triggers recruitment and assembly of multiprotein complexes that activate apical caspase 8 (ref. 28). The other principal death-signalling pathway involves the mitochondrion, which acts as an integrating sensor of multiple death insults by releasing cytochrome *c* into the cytosol where it triggers caspase activation. The mitochondrial pathway is thought to be the principal target of survival signalling pathways, which act by stabilizing mitochondrial function and integrity and suppressing release of cytochrome *c*[29]. Once cytochrome *c* has been released from the mitochondrion, it orchestrates assembly of an intracellular apoptosome complex that recruits apical caspase 9 via the adaptor protein Apaf-1 (ref. 30).

Viability of normal somatic cells requires survival signals that are idiosyncratic to each cell type; signals include soluble factors or direct physical interactions with neighbouring cells or ECM. Because such signals are available typically only within discrete somatic environments, metazoan somatic cells are in effect 'trapped' within specialized trophic microenvironments within the body, dying should they wander or become misplaced. Epithelial cells offer a particularly dramatic example of such somatic entrapment. Detachment from their neighbours or basal stroma triggers a spontaneous apoptotic suicide termed anoikis. In part, anoikis occurs because detachment deprives the cell of necessary integrin and cadherin-mediated survival signals. However, it has recently been shown that disturbances to the intracellular cytoskeleton induced by detachment can directly trigger apoptosis through release of pro-apoptotic BH3 proteins such as Bmf, which is normally kept inactive through binding to the actin-based motor complex (D. Huang, H. Puthalakath and A. Strasser, personal communication). Another BH3 protein, Bim, is bound to the LC8 cytoplasmic dynein light chain, which sequesters it to the microtubule-associated dynein motor complex, but is released in response to multiple apoptotic stimuli[31].

With such potent mechanisms in existence to obliterate displaced cells, it is no surprise that suppression of apoptosis is high on the list of acquired attributes in cancer cells. Known mutations in survival signalling pathways found in tumours include deregulated expression of the survival factors insulin-like growth factor (IGF)-I and

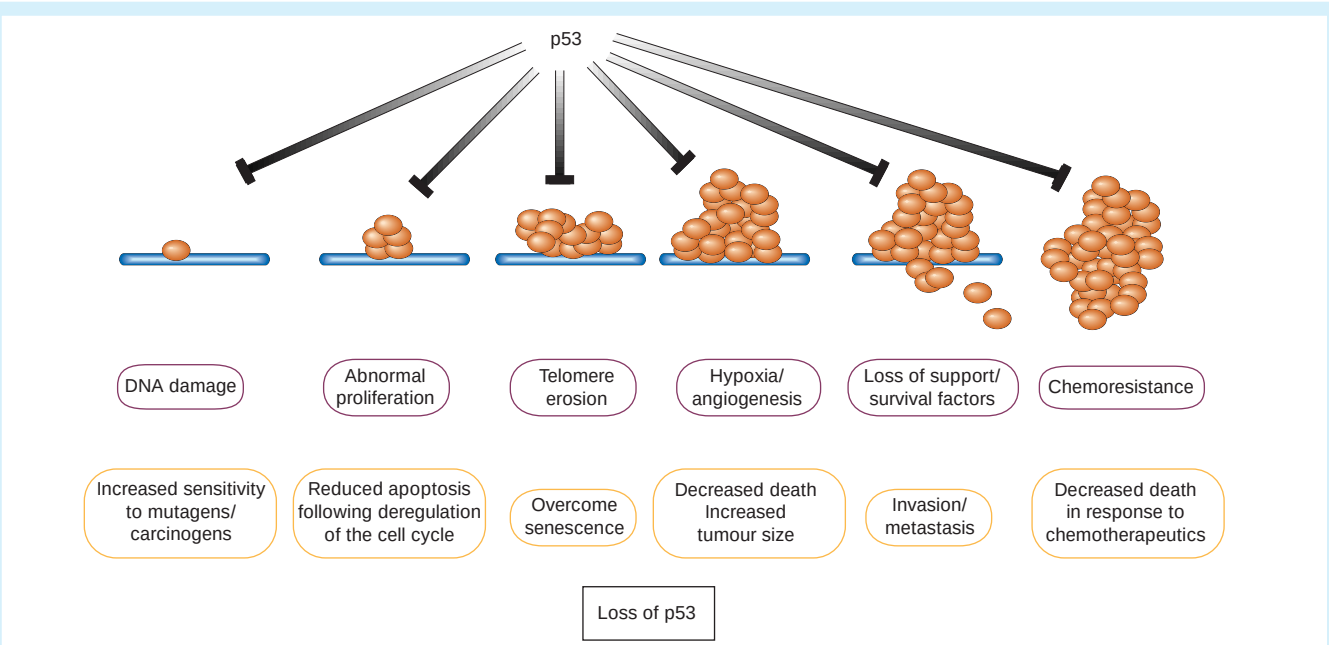

Figure 3 Many stress signals encountered during tumour progression activate p53, resulting in apoptosis or growth arrest. Loss either of the ability to activate p53 or of p53 function itself has considerable impact on the 'success' of the carcinogenic process, as it increases the chances of a tumour cell surviving progressively adverse conditions. Inability to activate p53 in response to stress signals encountered early during tumour development, such as deregulated proliferation, may to be sufficient to allow the formation of preneoplastic lesions. However, lesions that suppress activation of p53 in response to such oncogene-associated stress signals do not necessarily block activation of p53 by subsequent events encountered during malignant progression, such as DNA damage. Consequently, additional alterations in pathways that activate or respond to p53, or loss of p53 by direct mutation of the gene itself, may be selected during progression to more malignant cancers.

IGF-II (ref. 32), activating mutations of Akt, a serine/threonine kinase that induces a strong survival signal[33,34], and loss of the suppressor of Akt function PTEN[35–37]. The anti-apoptotic oncoproteins Bcl-2 and Bcl-x_L, which exert their principal effects through stabilization of the mitochondrion, are found to be overexpressed in several tumour types and recent analyses have indicated that loss of Apaf-1 is a relatively frequent event in malignant melanoma that presumably confers resistance to apoptosis[38].

A particularly potent driving force for the suppression of apoptosis in tumour cells is the coupled relationship between cell proliferation and cell death, a phenomenon exemplified by the Myc protein. In addition to its well documented growth-promoting property, Myc was found to be a powerful inducer of apoptosis, especially under conditions of stress, genotoxic damage or depleted survival factors[39,40]. Consideration of such observations led to the proposal that the innate apoptotic potential of Myc serves as an in-built foil to its oncogenic capacity (Fig. 2 and refs 39, 41, 42). Similar antagonistic duality has since been described for essentially all known growth-promoting proteins, including E2F1 (refs 43–46), whose pro-apoptotic activity provides a counter to the proliferative effect of loss of pRB[3]. Even under circumstances where apoptosis is not induced by activation of oncogenes such as E2F1 (ref. 47) or Ras[48–50], an irreversible cell-cycle arrest is triggered in its place, which serves as an alternate mechanism to forestall continued proliferation.

Growth-deregulating oncoproteins seem to promote apoptosis through the activation of several downstream pro-apoptotic effector pathways. For example, Myc has a profound effect on the mitochondrion, triggering release of cytochrome *c* and activation of caspase 9. This pathway is inhibited by members of the Bcl-2/Bcl-x_L anti-apoptotic family and by survival factors, both of which have been shown to potentiate the oncogenic action of c-Myc[51–55]. E2F1 can directly influence apoptotic signalling from death receptors[56], whereas Myc greatly enhances sensitivity to signalling through the CD95 (ref. 57), TNF[58] and TRAIL[59] death receptors. Another common pathway through which a wide variety of proliferative signals influence the apoptotic programme is through induction of ARF, an alternate product of the *INK4a* locus, one of whose functions is to trigger upregulation of p53 through its inhibitory action on MDM-2 (ref. 60). Yet another pathway recently described for Myc seems to involve rapid downregulation of E-cadherin, which may put the affected cell into a state of *de facto* anoikis (S. Pelengaris and G.E., manuscript in preparation).

Another potent selective pressure in cancers to suppress apoptosis arises from the fact that programmed cell death is the typical response of somatic cells to many forms of stress and damage; in particular damage to cell DNA (a fact exploited by most classical cancer therapeutics). Stress-associated signals that activate apoptosis include many of those encountered by the incipient tumour cell, including hypoxia and nutrient deprivation, as well as DNA damage arising from telomere erosion, defective repair, oncogene deregulation and therapy (see review in this issue by Hoeijmakers, pages 366–374). The p53 protein is important in transducing such diverse signals into tumour-suppressive apoptotic or growth-arresting responses, which implies that there is strong selection for tumour cells to loose p53 function[61]. Importantly, differing p53-activating stresses tend to arise at different stages of carcinogenic progression. For example, oncogene deregulation occurs early, as it is a prerequisite for clonal expansion, whereas hypoxia is significant only after the tumour reaches macroscopic size. Consequently, p53 exerts a tumour-suppressive role at multiple stages of carcinogenic progression (Fig. 3), offering an explanation for why loss of p53 has such a profound effect on tumour development.

But the notion that p53 is a cellular superhero that functions solely to protect the organism from itself is almost certainly too simplistic. In those systems where tumour progression can be followed from pre-malignancy through to invasive cancers, p53 mutation is seldom one of the earliest events. For example, in both mouse skin carcinogenesis[62] and human colon cancer development[63], mutation of p53 occurs at the point of transition from pre-malignant to invasive lesions, well after activation of some of the oncogenes that are thought

to trigger the p53 response. One probable reason for this is that alternative mutations in early-stage tumours serve to incapacitate some aspects of the p53 response. The best described of these affects ARF, whose loss severs the link between deregulation of oncoproteins such as Ras, Myc and E2F, and consequently p53 activation, permitting cells to proliferate and survive in the face of oncogene deregulation. Although mutations specifically altering the ARF protein are uncommon in human cancers, other mechanisms that hinder ARF function have been described, including methylation of the ARF promoter and amplification of genes such as *Bmi-1* (ref. 64), *Twist*[65] and *TBX2* (ref. 66), which encode repressors of ARF expression.

Inactivation of ARF through methylation of the ARF promoter occurs in both carcinomas and adenomas of the colon[67,68]. This probably confers on colonic enterocytes the capacity to continue to proliferate despite activation of Ras, a situation that may be further exacerbated by the ability of Ras to induce expression of the p53 inactivator MDM-2 (ref. 69). But although loss of ARF serves to suppress the p53 response to oncogene activation, it leaves p53 available within the cell to respond to other ARF-independent stress. Ultimately, the evolving cancer cell will still run into a p53-induced block, at which point inactivation of p53 may be the only mechanism by which the tumour cell can endure. Of course, such a model begs the question: why is p53 not mutated in early pre-malignant lesions, as this would presumably strip the cell of any opposition to malignant progression? One possibility is that ARF possesses p53-independent tumour-suppressive activities that are independently selected against in early neoplasias. Another intriguing notion is that loss of p53 could confer some kind of immediate selective disadvantage upon the affected cell that must be overcome before the tumour can progress further. This idea is supported by surprising experimental data indicating that *p53*-null mice are less susceptible to development of carcinogen-induced papillomas[70–72]. However, once neoplastic lesions do arise in such mice, albeit at greatly reduced frequency, their progression to invasive carcinoma is more or less immediate.

Not only can p53 loss have different effects at various stages of carcinogenesis, but it can also have far-reaching consequences for the evolutionary trajectory of tumour progression by transforming potent tumour-suppressive mechanisms into powerfully oncogenic ones. For example, erosion of telomeres in aberrantly proliferating cells generates a powerful DNA damage signal that triggers p53-dependent growth arrest and apoptosis, and efficiently ablates potential tumour cells that exhaust their proliferative potential. However, cells that lack functional p53 are unable to respond in this way and are forced to endure the catastrophic consequences of telomere erosion, resulting in 'rampant genome instability'[14,73]. Similarly, oncogenic consequences of defective DNA-repair machinery are probably minimal in p53-positive cells that can respond appropriately to damaged DNA. By contrast, the combination of compromised repair (a process to which p53 also contributes) together with suppressed apoptosis is likely to constitute a heady oncogenic brew.

Restraints to the acquisition of heritable diversity

As already described, cancer development depends on the acquisition and selection of specific characteristics that set the tumour cell apart from normal somatic cells. It is thought that most cancer is precipitated by *de novo* mutations in somatic cells, a process that may be accelerated by the genomic instability inherent to most cancers[74]. However, the extent to which genomic instability is a prerequisite for tumour development remains unclear, as to some degree the chromosomal chaos characteristic of almost all tumour cells may be merely be an indicator of some past acute genome-destabilizing event, such as telomere erosion. Moreover, the requirement for new mutations to drive tumour progression may be partly substituted by loss of mechanisms that limit the phenotypic expression of innate genetic variation that is inherent to all cells. Loss of HSP90, for example, has been shown to reveal extensive morphological variation that is usually silenced[75]. The existence of protein variability that is normally buffered through protein-polishing mechanisms like HSP90 leads to the possibility that release of this innate variation may complement, and to some degree substitute for, the requirement for new somatic mutations during tumour development.

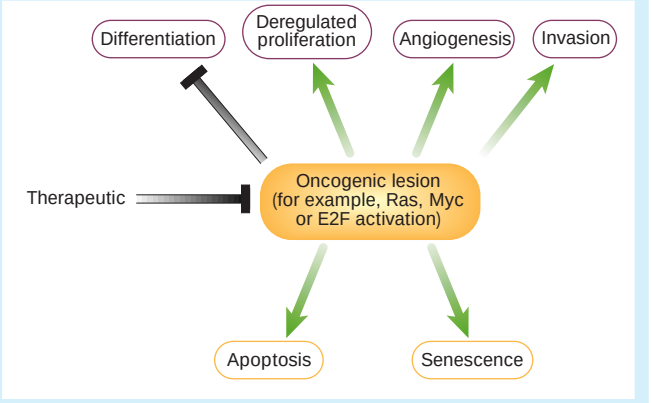

Figure 4 Growth deregulating lesions generate profound, diverse and cell-type specific pleiotropic changes in a cell and its surrounding. Some of these (proliferation, angiogenesis, suppression of terminal differentiation, local invasion) augment the neoplastic effect of the primary lesion, whereas others (sensitization to apoptosis, induction of growth arrest or senescence) are innate defences that inhibit it. Inhibiting the primary growth-deregulating lesion will influence all of these downstream sequelae. The net result is not straightforward to predict and will vary depending upon the cell type affected and the composition of lesions driving the particular neoplasm.

Therapeutic targeting of cell proliferation and apoptosis

Because deregulated proliferation and inhibition of apoptosis lie at the heart of all tumour development, they present two obvious targets for therapeutic intervention in all cancers. Clearly there are numerous mechanisms through which these two defects can occur, and the success of targeted therapy will depend to a large part on the molecular fingerprinting of individual tumours.

Although most existing cancer drugs are anti-mitotic, they act not by targeting the specific lesions responsible for deregulated tumour growth, but by crudely interfering with the basic machinery of DNA synthesis and cell division. Moreover, we now know that the surprising selectivity of such crude agents results largely from the increased sensitivity to apoptosis afforded to tumour cells by their oncogenic lesions[3,39,76]. Drugs designed to specifically inhibit growth-deregulating lesions are currently being tested in clinical trials, and include inhibitors of RTKs, Ras, downstream signalling kinases such as the mitogen-activate protein kinase and Akt pathway, and CDKs[77].

At first glance, targeted inhibition of growth-deregulating lesions in cancer would be seem to have limited therapeutic efficacy, as they would at best be cytostatic. However, unexpected therapeutic bonuses may emerge from such an approach because growth deregulation induces a plethora of downstream activities in affected cells and their adjacent tissues. For example, growth-deregulating lesions such as E2F and Myc are potent inhibitors of differentiation in many cell lineages. Therapeutic inhibition of the offending oncoprotein in tumours arising from cell lineages where terminal differentiation has been blocked could be sufficient to trigger a resumption of that differentiation programme, permanently expelling the tumour cell from the proliferating compartment. Such ideas receive support from several *in vivo* mouse models. For example, in skin tumours induced by deregulated Myc expression, subsequent inactivation of Myc leads not only to cessation of proliferation, but also to the expeditious resumption of normal keratinocyte differentiation which rapidly becomes irreversible[24]. A similar resumption of terminal differentiation pathways is also observed after removal of the Myc signal in Myc-induced T-cell lymphomas[78].

Another direct consequence of certain oncogenic lesions is angiogenesis. Both activated Ras and deregulated Myc are potently angiogenic, suggesting that their pharmacological inhibition might foster the collapse of tumour vasculature. In a reversible Ras-dependent mouse model of melanoma, inactivation of Ras triggers the rapid involution of tumour vasculature, with concomitant regression of the tumour[79]. Similarly, Myc has potent angiogenic capacity that has been observed in skin[24], pancreatic β cells (S. Pelengaris and G.E., unpublished data), lymphoma[80], neuroblastoma[81] and in a fibroblast xenograph model[82]. Myc directly induces angiogenesis without any apparent need for an angiogenic switch, in part by induction of vascular endothelial growth factor (VEGF)[24] and possibly downregulation of the angiogenesis negative modulator thrombospondin-1 (ref. 83). Importantly, Myc-induced angiogenesis is of the leaky, immature and unstable kind so often associated with neoplasia. And, as seen in the Ras model system, inactivation of Myc in switchable Myc transgenic models of skin and β cells leads to rapid regression of tumour vasculature, triggering concomitant tumour involution (ref. 24, and S. Pelengaris and G.E., unpublished data).

Such studies offer encouragement for the idea of therapies based around specific targeting of the cell's proliferative machinery. However, anti-proliferative therapeutics need to be approached with caution. As outlined above, growth-deregulatory mutations trigger pleiotropic and tissue-specific effects, some of which serve to enhance the malignant state (proliferation, angiogenesis, suppression of differentiation), whereas others (sensitization to apoptosis) suppress it (Fig. 4). As these would all be inhibited by a single agent that blocks the initiating growth-deregulatory lesion, the therapeutic consequences of such an agent are likely to be highly tissue- and tumour-specific and, at present, difficult to predict.

The second obvious strategy for cancer therapy is to target the lesions that suppress apoptosis in tumour cells. The potent pro-apoptotic effects of growth-deregulating mutations mean that tumours are peculiarly dependent upon their particular suite of anti-apoptotic mutations for continued survival. Thus, although apoptosis in tumour cells is sufficiently suppressed to below a critical threshold to enable them to survive, they remain acutely sensitized to apoptosis. In most, if not all, cancer, this ability to survive results in part from inhibition of the p53 pathway, either by inactivating mutations in p53 itself, perturbation of the signalling pathways that allow activation of p53 in response to stress, or defects in the downstream mediators of p53-induced apoptosis. Reintroduction of p53 function is sufficient to induce apoptosis in many tumour cells, and several mechanisms to reactivate p53 are being considered as therapeutic strategies. These include introduction of wild-type p53 into tumours expressing a mutant protein, or inhibition of negative regulators of p53, such as MDM-2, in those tumours that retain wild-type p53 (ref. 61).

Interference with survival signalling is another appealing approach to the induction of apoptosis in tumour cells, either by direct inhibition of components of the signalling cascades, such as STI571 inhibition of Brc-Abl in chronic myelogenous leukaemia[84], or by inhibition of angiogenesis by drugs that target the VEGF receptors Flt-1 and KDR[85]. Reintroduction of inhibitors of VEGF expression, such as VHL, also represent interesting targets in this context[86]. Direct participants of apoptotic pathways, such as the Bcl-2 proteins that are important in both cancer development and the acquisition of resistance to conventional cancer therapies, provide further targets for the development of drugs that may be indifferent to the p53 status of the tumour cell[87].

Regardless of efficiency in cell killing, the success of repairing the apoptotic response in tumour cells depends on the extent to which such therapies confine death to the cancer cells, and allow survival of normal tissue. Many conventional chemotherapies induce significant toxicity, particularly in tissues that normally maintain a proliferative compartment, such as gut epithelium and the haematopoietic system. This DNA damage-induced toxicity is mediated in part through p53, leading to the suggestion that inhibition of p53 in these normal tissues may protect against drug-induced toxicity, thereby improving the tolerance of conventional cancer therapies[88]. However, implicit in the development of drugs that target specific lesions responsible for tumour cell growth is the prediction that these approaches will show significantly more specificity for tumour cell killing than conventional therapies.

Although activation of apoptotic pathways can lead to the death of untransformed cells, a process that is essential in normal development, a fundamental difference exists between tumour cells and their normal counterparts, as normal cells neither have to sustain the pro-apoptotic onslaught that is inherent in deregulated proliferation, nor survive away from their usual environment in the absence of requisite survival signals. Repair or replacement of a single apoptotic signal, be it reactivation of p53 or removal of a survival signal, could well prove too much for a tumour cell already burdened with a heavy apoptotic load. By contrast, the same perturbation may scarcely ruffle the equilibrium of a normal cell, safely buffered in its appropriate soma and enjoying the full gamut of trophic support that ensures normal cell survival. An interesting variation on this theme is illustrated by the activity of antagonists of Cdk2. These inhibitors, which would ostensibly function to prevent cell-cycle progression, prevent normal phosphorylation and inactivation of E2F1 at the completion of DNA synthesis. The outcome is tumour-specific apoptosis, presumably stemming from an inability of tumour cells to tolerate yet further deregulation of E2F activity, beyond that already sustained through perturbation of the pRB pathway[89]. Whether this difference between normal and tumour cells actually exists in a meaningful way, and whether we can fully exploit it in the development of new drugs to treat cancers, are questions and challenges that now face us.

Clearly, all forms of tumour therapy carry with them the danger of selection for resistance, a problem that may be exacerbated by the genomic plasticity inherent in most, if not all, cancers. The most effective solution to this problem is almost certainly to simultaneously attack multiple lesions specific to individual tumours, in a much more sophisticated version of standard combined chemotherapies used at present. Evolution of cancer therapy is likely to remain a combination of design and error, but the development of mechanisms to target the mission-critical events that are common to all cancers provides a glimpse of therapeutic potential hitherto unimaginable. □

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