Apoptosis and carcinogenesis

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Many tumours are characterised by increased levels of apoptosis. This observation establishes significance for this process in tumour development, but it does little to elucidate the nature of this role, nor does it yield information relevant to the early stages of carcinogenesis. To gain a better understanding of the importance of apoptosis, it has been necessary to create a number of transgenic model systems wherein the apoptotic response has been modified. Using this strategy, a number of genetic lesions have been identified which affect both the apoptotic pathway and predisposition to malignancy. These lesions can operate either directly, by blocking the induction of apoptosis; or indirectly, by increasing the selective pressure for further genetic change. The consequent deregulation of growth control and increase in mutation burden represent two key steps in carcinogenesis, underlining the pivotal role played in tumour suppression by the normal induction of apoptosis.

The homeostatic maintenance of cell number within a tissue is finely balanced between the active processes of cellular proliferation and cell death, with conversion to malignancy typically characterised by lesions in genes intrinsic to both of these processes. The emerging association between genetic lesions, apoptotic dysfunction and tumourigenesis has provoked a closer examination of the role played by apoptosis in tumour prevention. Such studies have helped to define apoptosis as a feature of both normal and malignant tissue, particularly in response to DNA damage. This finding has clear relevance to the therapy of cancer, as many chemotherapeutic drugs function by inducing apoptosis. A number of oncogenes and tumour suppressor genes are now known to regulate cell death. Elevated or ablated expression of these genes can be shown to influence the apoptotic response and, usually through synergy with a variety of other growth promoting lesions, to result in tumourigenesis. The purpose of this review is to discuss the in vivo relevance of apoptosis in the prevention of cancer and also to explore the underlying genetic events responsible for the impairment of cell death.
c-Myc

Elevated expression of c-Myc occurs at a high frequency in many diverse tumour types. By implication, a lesion which results in over-expression of c-Myc must be an important event in malignant conversion. In vitro experiments designed to gain an insight into this process have suggested that the primary function of c-Myc is to promote cell division. Inhibition of c-Myc expression can be shown to block cellular proliferation in both haematopoietic and fibroblast cultures. Over-expression of c-Myc within cultured fibroblasts is sufficient to drive both normally cycling and quiescent cells into a continuous proliferative state, wherein they no longer respond to normal growth arrest signals, and will even continue to proliferate when deprived of mitogenic stimulation.

Wild type fibroblasts cultured in low serum conditions show c-Myc down-regulation, with cells entering growth arrest at the G1 stage of the cell cycle. However, if fibroblasts or haematopoietic cells are engineered to over-express c-Myc and subsequently cultured in low serum, no increase in cell number is seen, despite a marked increase in the rate of proliferation. Subsequent analysis of these cultures revealed a dramatic increase in the rate of apoptosis, resolving this apparent discrepancy and demonstrating a role for c-Myc in the regulation of cell death.

One prediction from these data is that a single lesion arising in an otherwise wild-type cell, resulting in the ectopic expression of c-Myc, is probably insufficient for cell transformation in vivo. Despite c-Myc conferred hyperproliferation, within an in vivo system where growth factors are limiting, the accompanying high level of c-Myc dependent apoptosis would attenuate clonal expansion or even remove such cells from a tissue as they arise. Thus, if the effect of elevated expression of c-Myc is considered in isolation, it may almost be viewed as self-limiting.

Such a view of the relevance of c-Myc is, however, clearly flawed, as c-Myc mutations are associated with tumourigenesis. The basis for this predisposition must, therefore, derive from synergy with other genetic lesions. For example, over-expression of c-Myc within a cell bearing mutations in pathways influencing either cell growth or apoptosis will lead to a loss of equilibrium between these two pathways. The consequences of such dissociation are either deletion, extended survival, or hyperproliferation. The latter two eventualities will clearly predispose a cell to malignant conversion.

The generation of transgenic mice over-expressing c-Myc has provided an opportunity to examine the above predictions and to test directly the influence and relevance of c-Myc dependant apoptosis to the processes of in vivo tumourigenesis.

Burkitt’s lymphoma, a human malignancy of B cells, is characterised by a chromosomal translocation at the c-Myc locus. Transgenic mice,
characterised by c-Myc over-expression, have been designed to mimic this event (E μ-Myc mice). B-cell lymphomas were observed in 90% of these animals after a latency period of approximately 5 months\(^9\). During this latency period, increasing numbers of large proliferating B lymphocyte lineage cells were detected in the blood, but very high levels of apoptosis were also detected. Analysis of the bone marrow at the time when the tumour had developed still showed high levels of apoptosis within the tissue.

These experiments support the idea that c-Myc drives tumourigenesis in combination with other genetic changes, and further show that these events occur within an environment characterised by high levels of proliferation and apoptosis. Indeed, the tumourgenic effects of c-Myc may be considered to arise as a direct consequence of increased cell turnover, increasing the rate at which different mutation bearing cells cycle through any given population, independent of actual mutation frequency. By exposing the cell population to a greater range of mutations, c-Myc over-expression therefore places selective pressure upon mutations which will block the elimination of cells through apoptosis.

The B cell lymphocytes of the E μ-Myc mice are, therefore, in a c-Myc conferred state of rapid cell turnover. Secondary lesions will arise within these cells, which will normally be removed via apoptosis. However, once a lesion arises which blocks apoptosis, this cell will be strongly selected for, expanding with the potential to develop into a tumour. Presumably the variability in latency period between individual E μ-Myc mice reflects the time required for the appropriate secondary lesion or lesions to arise.

Since these first observations, a range of c-Myc transgenic mice have been generated, underlining the ability of this gene to predispose to tumourigenesis in synergy with other apoptosis suppressing lesions, such as TGF alpha\(^10,11\). Recently, the emergence of inducible systems has made possible somewhat more controlled analysis of the in vivo effects of c-Myc over-expression. Using a c-Myc estrogen receptor fusion protein, c-Myc activity can be rendered inducible upon addition of the drug 4-hydroxytamoxifen. By generating rat-1 fibroblast cultures transgenic for this construct, and subsequently tumours within SCID mice, c-Myc activation was shown to lead to the induction of apoptosis, especially in regions of hypoxia, and in a manner influenced by Bcl-2\(^12\).

**Bcl-2**

The Bcl-2 gene was initially cloned and identified as a candidate proto-oncogene involved with the t(14:18) translocation characteristic of the
human B-cell malignancy, follicular lymphoma. In vitro analysis of Bcl-2 showed this gene to differ in function from conventional oncogenes, failing either to promote growth or directly lead to cellular transformation. Vaux, in 1988, first argued that the Bcl-2 gene functions by inhibiting cell death. By utilising a haematopoietic cell line, dependent on the cytokine interleukin 3 (IL-3), he demonstrated that the over-expression of Bcl-2 conferred survival, but not growth, when cultured in the absence of IL-3.

Several murine transgenic strains have been generated to test directly the biological relevance of Bcl-2 over-expression in vivo. One strain has been engineered to express a Bcl-2 transgene under the influence of an immunoglobulin enhancer (Bcl-2-Ig). This construct drives over-expression specifically to B lymphocytes, in theory generating a murine model of human follicular lymphoma.

The Bcl-2-Ig mice exhibited a polyclonal follicular expansion of small resting B cells. As these cells are non-proliferative, it was concluded that the increase in cell number was due to enhanced cell survival, a direct result of suppression of apoptosis by Bcl-2. Following a latency period of approximately 1 year, 15% of the Bcl-2-Ig mice developed aggressive B cell lymphomas, analysis of which revealed elevated levels of c-Myc expression in approximately 50% of tumours.

As with the case of the E mu-Myc mouse, the long latency period implies a requirement for additional genetic lesions prior to tumourigenesis. In this situation, it is argued that elevated Bcl-2 expression leads to impaired apoptosis and thus selective pressure on mutations in genes controlling cellular proliferation, including c-Myc. Such synergy between these two genes has been directly shown in vitro. As illustrated by the Bcl-2-Ig mouse, when two lesions arise within a cell, one conferring impaired apoptosis, the other rapid cell turnover, the resultant synergy can promote rapid clonal expansion leading to progressively increased genomic instability and ultimately to malignancy.

The idea that a strong in vivo synergy exists between lesions conferring over-expression of both c-Myc and Bcl-2 has gained further support from data derived from mice over-expressing both of these genes. Analysis of the resulting offspring highlighted a strong predisposition to rapidly emerging undifferentiated haematopoietic leukaemia.

\[ p53 \]

Loss of function of the tumour suppressor gene p53 is strongly associated with the development and progression of many human and murine tumours. Wild-type p53 gene function has been demon-
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strated to be a critical regulator of both the cell cycle and apoptosis in response to genotoxic insult. Normal cells in culture, when treated with agents that cause DNA strand breaks (i.e., UV light\textsuperscript{22}, ionising radiation\textsuperscript{23} and selected chemotherapeutic drugs\textsuperscript{24}), accumulate p53 protein and growth arrest or die. p53 also mediates apoptotic induction following hypoxia (e.g., regions of low oxygenation at the centre of poorly vascularised solid tumours), such that cells bearing wild type p53 will be more efficiently removed from malignant tissue than p53 \(-/-\) cells\textsuperscript{25}.

p53 mediates its effect upon the cell cycle and apoptosis both directly through protein:protein interactions and indirectly by transcriptional regulation of a range of downstream target genes. Indeed, a plethora of different functions are now ascribed to p53. In summary, p53 influences several essential processes aimed at the prevention of mutant cell propagation. It appears to have both a direct role in DNA repair and also in the regulation of DNA repair enzymes, a role in cellular growth arrest and in inducing apoptosis following exposure to genotoxic stress. It is, therefore, possible to make several predictions about the effect of p53 deficiency upon both mutation rate and tumourigenesis. The availability of strains of p53 deficient mice\textsuperscript{26-28} has permitted a range of experiments addressing both of these endpoints.

**p53 and mutation frequency**

Abrogation of the p53 dependent apoptotic pathway is predicted to lead to an increase in the number of cells bearing DNA damage, as these would normally be deleted. Experiments designed to test this prediction suggest that p53 dependent differences may only become apparent following exogenous DNA damage. Two different groups have used a transgene target to monitor spontaneous mutation frequency, but in both cases no p53 dependent difference was observed\textsuperscript{29,30}. By contrast, experiments using either short term pre-B cell cultures\textsuperscript{31} or a transfected fibroblast line\textsuperscript{32} have now provided data in support of a p53-dependent increase in the number of mutation bearing cells following genotoxic stress. *In vivo* experiments using the Dlb-1 locus as a marker for mutation, have also shown a p53 dependent increase in mutation frequency in the murine small intestine\textsuperscript{33}, although this was only observed at high levels of DNA damage. Taken together, these different experiments do support a role for p53 in preventing the acquisition of mutation, but it is clear that it is not the sole mechanism, particularly at low levels of DNA damage.

A further difficulty exists in interpreting the relevance of p53 dependent apoptosis because of the diversity of roles played by p53.
However, evidence does exist to show that, at least in some cell systems, the induction of apoptosis is the critical response to DNA damage. Thus, where mutation frequency and clonogenic survival have been scored independently, it can be shown that mutation frequencies amongst surviving clones are largely unaffected by p53 status, but that the effect of p53 deficiency is to increase the number of mutation bearing progeny by virtue of increased cell survival. The end result of this process is, therefore, to increase the yield of DNA-damage bearing cells, as a direct consequence of failure to engage apoptosis.

**p53 deficiency and in vivo tumourigenesis**

The majority of p53 —/— mice develop normally to birth, but then rapidly succumb to tumourigenesis, predominantly of the thymic lineages. p53 +/- mice develop both lymphomas and sarcomas at an approximately equal rate. The +/- mice develop a broader spectrum of tumours at low incidence in comparison to the —/— mice and also exhibit a longer latency period before the appearance of a tumour. Transgenic experiments using a dominant negative p53 transgene in combination with the p53 null animals have strongly supported the idea that this type of mutation predisposes to tumourigenesis by blocking wild type p53 function.

In vivo analysis of these strains has been used to address the relevance of p53 gene function at different stages of tumourigenesis. Both Kemp et al and Lee et al have examined the in vivo consequences of exposure to ionizing radiation on mice mutant for p53. Ionising radiation is a powerful inducer of p53-dependent apoptosis, and the doses used in these experiments normally elicit a strong apoptotic response. Both studies reported data supporting a critical role for wild type p53 in preventing radiation induced malignancy. Using p53 null animals and mice transgenic for a dominant negative p53, Lee et al showed a higher incidence of sarcoma and lymphoma, and also demonstrated an increase in the number of double strand chromosomal breaks. Similarly, Kemp et al found that treated +/- and —/— mice developed tumours with an identical spectrum to non-irradiated counterparts, but following a much reduced latency period.

Analysis of tumours derived from irradiated heterozygous mice revealed loss of the remaining wild type p53 allele in 96% of all cases, a significantly higher level of loss than in the mock irradiated +/- mice. Loss of p53 function is, therefore, strongly selected for during this process. These data show that p53 status is not the sole determinant of the rate of tumourigenesis, as p53 —/— mice do not develop large
numbers of primary tumours following irradiation, even although p53 is absent from every cell.

The protective role of p53 is not limited to the prevention of γ-irradiation mediated tumourigenesis. For example, treatment of p53 +/— animals with the carcinogen dimethylnitrosamine leads to a reduction in latency (compared to wild type) in tumour development within the liver.

p53 status has also been shown to influence both the initiation and progression of UV induced skin cancer. Ziegler et al. have demonstrated that the majority of actinic keratoses (first clinically apparent lesion in skin cancer development) have at least one mutated p53 allele. To further examine the role of p53 status in these lesions, mice +/+, +/— and −/− for p53 were subjected to UV-B irradiation. After 24 h, +/+ mice exhibited extensive sunburn, characterised by high levels of apoptosis. Significantly, −/− skin tissue presented far fewer sunburn cells and correspondingly less apoptosis. +/− skin tissue exhibited an intermediate phenotype.

Based on these observations Ziegler et al. concluded that p53 +/− and −/− cells possess a selective survival advantage over +/+ cells upon repeated re-exposure to sunlight, as the ability to become sunburn cells and enter apoptosis is clearly p53 gene dose dependent. Cells surviving by virtue of compromised p53 activity, which subsequently acquire lesions in growth or death regulating genes, will be predisposed to malignancy. Hence, p53 deficiency strongly promotes malignancy in this system, wherein UV irradiation is acting as both tumour initiator and promoter.

Skin swabbed with the carcinogen DMBA initiates tumourigenesis in the mouse. Promotion of tumourigenesis can be achieved by exposing the initiated skin to TPA, the result of which is the appearance of multiple papillomas (benign hyperplastic regions). Within 2 months, a small proportion of these papillomas become carcinomas, which vary in their state of differentiation and invasiveness. Kemp et al. utilised this well characterised model of chemically induced multi-stage tumourigenesis to determine the effects of p53 status upon these different stages.

p53 −/− mice were swabbed with the tumour promoter TPA. No papillomas arose from this treatment, showing that loss of p53 function is insufficient for tumour initiation. Indeed, these data show that, contrary to expectation, a p53 null phenotype is refractory to papilloma formation. The effect of p53 status on the latency of papilloma development was also investigated and no difference was observed between the +/+, +/− and −/− mice, again implying a role for other genetic lesions at this stage of tumour development.

The conversion rate from benign papillomas to malignant carcinomas was, however, shown to be highly dependent upon p53 status. In p53
null mice, conversion occurred at higher penetrance and significantly faster than in +/+ animals. These somewhat contradictory results are supported by experiments using transgenes epidermally targeted to express v-Ha-ras, v-fos and human growth factor alpha. These transgenic strains are predisposed to papilloma development, but this is again blocked by crossing onto a p53 null background\textsuperscript{41}. Taken together, these experiments argue that failure of p53-dependent pathways leads to increased genomic instability and, therefore, increased rate of malignant progression, but only beyond the point of papilloma development. Prior to this point, it is possible that cells can tolerate a higher level of DNA damage by virtue of attempted repair by p53 dependent pathways, whereas damaged p53 null cells are deleted by p53 independent apoptotic mechanisms. These experiments highlight the potential difficulty in over-interpreting the role of apoptosis, as in this system failure to invoke p53 dependent pathways actually confers protection, at least against the benign lesion.

p53 deficiency clearly plays an important role in malignant development yet, as has been shown above, it is not the sole determinant of this process. The nature of the complex inter-relationship between p53 and other tumour related genes has been investigated through a range of transgenic intercrosses. These experiments have produced results which, while strongly supporting a tumour suppressor role for p53, clearly underline the cell specific nature of this function. For example, mice transgenic for an MMTV driven c-Myc transgene predominantly develop mammary carcinoma, yet crossing onto a p53 null background reveals synergy in tumour development within the haematopoietic system, with no effect upon mammary tumourigenesis\textsuperscript{42}. Further evidence is available for synergy between p53 and c-Myc within the lymphoid system, as mice null for p53 and transgenic for a CD2 driven c-Myc construct show accelerated lymphoma development\textsuperscript{43}.

Intercrossing the p53 null mice with animals heterozygous for the Apc tumour suppressor gene has yielded similarly contradictory results. Mice heterozygous for Apc normally develop adenomas of the small and large bowel. When crossed onto a p53 null background, no difference in tumour formation or progression was observed\textsuperscript{44}, even though loss of function of p53 is strongly implicated in intestinal neoplasia. Synergy between these mutations was, however, observed within the pancreatic tissues in the intercrossed animals, with almost 100% of doubly mutant mice developing neoplasia.

In summary, the experiments discussed above argue strongly for complex reliance upon p53-dependent pathways, largely determined by cell type. However, this may again be too simplistic a view of p53 function as, even within a given tissue type, deficiency of p53 can lead to
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different endpoints. For example, as stated above, p53 fails to synergise with c-Myc in mammary carcinoma development, yet it does accelerate mammary adenocarcinoma formation in conjunction with a Wnt-1 transgene\textsuperscript{45}.

These differences may arise as a consequence of other genes modulating apoptosis. For example, data from intercrosses of Bcl-2 transgenic animals and p53 null mice strongly argue that p53 deficiency may be substituted for by Bcl-2 gene deregulation\textsuperscript{46}. One alternative explanation for the variable reliance upon p53 derives from the multiple functions of this gene. If one presupposes that tissue types vary in their reliance upon apoptosis or DNA repair to eliminate mutation, it is inevitable that there will be a pattern of tissue specific reliance upon p53 and, indeed, any other genes which regulate these processes. Finally, it should again be stressed that, although it is possible to detect a p53-dependent apoptotic phenotype within many of the systems discussed here, it is currently not possible to determine if this is the sole mediator of phenotype. The future analysis of p53 point mutants may enable the dissection and analysis of specific p53 dysfunction and their phenotypic consequences. It seems more likely, however, that it is the lack of a combination of p53 functions which ultimately determines predisposition to malignancy.

\textbf{pRb}

Loss of Rb gene function is frequently associated with tumours of the lung, breast, prostate and bladder in humans. Also, human Rb heterozygotes develop retinoblastoma at almost 100\% incidence and, to a lesser extent, osteosarcomas, predominantly during their early childhood. The rate limiting step in the development of these malignancies appears to be loss of the remaining Rb allele\textsuperscript{47}.

pRb is a 105 kDa phosphoprotein which has been shown to directly prevent the G1 to S phase transition of the cell cycle via the sequestration of the E2F family of transcription factor complexes. Rb's interaction with E2F inhibits the transcriptional upregulation of specific downstream genes, the function of which are deemed to be essential for the onset of the extensive DNA synthesis characteristic of S-phase in the cell cycle.

This model of Rb interaction is complicated by the presence of multiple E2F transcription factor family members within each cell, all of which can associate with pRb and the pRb family proteins (p107 and p130), with different affinities at different stages of G1\textsuperscript{48}. The precise extent of functional overlap between the different members of the pRb
family is as yet unclear, as is the potential for p107 and p130 to act as tumour suppressors. Data relevant to this point come from experiments analysing the p107 and p130 knockout mice, which have shown a primary role for these genes in regulating embryonic limb development\textsuperscript{49,50}.

By indirect implication, therefore, pRb appears to be a downstream mediator of p53 dependent growth arrest at G1. Recent experiments have identified one of the genes responsible for mediating this interaction, namely p21. Expression of p21 is induced in a p53 dependent fashion following DNA damage\textsuperscript{7,51} and p21 is a potent inhibitor of the family of proteins responsible for the increase in phosphorylation of pRb, the cyclin dependent kinases (CDKs)\textsuperscript{52}. Upon p21 induction, the CDK proteins are functionally suppressed and no longer able to increase the degree of phosphorylation on pRb. pRb in turn remains associated with the E2F transcription factor complex thus holding the cell in a reversible G1 arrest\textsuperscript{53}.

The predicted \textit{in vivo} phenotype following loss of Rb gene function is one of dysregulated proliferation of both normal and DNA damaged cells, with an expected predisposition to malignancy. The production of mice bearing a targeted disruption of the Rb allele has provided a powerful resource for investigating the \textit{in vivo} consequences of Rb loss.

The absolute requirement for Rb gene function during embryogenesis was made clear, when attempts\textsuperscript{54-56} to breed targeted Rb heterozygous mice to homozygosity resulted in embryonic lethality between days 13.5 and 15.5 p.c. Dying embryos are characterised by a disorder of early haematopoiesis and extensive cell death within the developing central nervous system. Rb +/- mice are viable, but do not develop the characteristic retinal malignancies associated with RB +/- humans, instead developing tumours of the intermediate lobe of the pituitary gland. This difference between human and mouse is further underlined by the failure to observe RB loss in human pituitary carcinomas\textsuperscript{57}.

To further investigate the \textit{in vivo} tissue requirements of Rb gene function, Rb +/- <-> -/- chimeric mice were produced\textsuperscript{58,59}. These animals were generated from Rb +/- blastocysts and Rb -/- embryonal stem (ES) cells. Despite the observed embryonic lethality in the pure bred Rb -/- mice, all tissues examined in the chimeric mice contained a proportion of viable Rb -/- cells. Tumourigenesis was only associated with the pituitary gland, with all resultant tumours genotyped as Rb -/-.

As all Rb -/- cells theoretically possess the capacity for unrestrained proliferation, the failure to observe tumour development outwith the pituitary gland appears paradoxical. One explanation for such an apparent discrepancy may lie in the apoptotic phenotype of Rb -/- cells. Indeed, examination of Rb chimeric animals revealed both aberrant cellular proliferation and increased apoptosis, especially in
the retina and lens of these animals. It has been postulated, therefore, that any dysregulated proliferation conferred by Rb deficiency is normally restrained through apoptotic mechanisms, and tumourigenesis is, therefore, dependent on at least one other genetic step. However, this apoptotic ‘safety net’ seems either not to be present or not as efficient in some tissues, including murine pituitary cells and human retinal cells, where other genetic lesion are not required before tumourigenesis can occur.

The genetic control over this ‘safety net’ response has been at least partially studied in experiments using animals mutant for both Rb and p53, which have shown that some of the observed apoptosis in the Rb null animals is p53 dependent. Doubly null embryos no longer exhibit extensive cell death in the developing lens and undifferentiated lens fibre cells. Data obtained from Rb +/-, p53 -/- animals also supports a significant role for p53 in limiting the expansion of Rb -/- cells, as these animals develop additional tumour types to those observed in either individual genotypic background. These lesions include islet cell tumours, bronchial epithelial hyperplasia, retinal dysplasia and pineoblastomas (a tumour type related to retinoblastoma and occasionally observed in RB +/- humans).

A role for p53 is further supported by the phenotype of mice expressing the HPV E7 protein in the retina, which inactivates pRb. On a wild type background, these animals exhibit degeneration of the retina. However, if crossed onto a p53 null background, these animals develop retinoblastoma60. Similarly, murine tumours of the choroid plexus induced by the expression of a pRb binding fragment of the SV40 large T antigen grow slowly, with high levels of apoptosis within the tumour. Once crossed onto a p53 null back-ground, apoptosis is diminished and tumour development is more rapid61.

As discussed above, loss of Rb function, appears to predispose to malignancy in a similar way to c-Myc over-expression, namely by placing greater selective pressure on other mutations affecting cell death. Recent work suggests that this is not the only way in which Rb deficiency can lead to tumour development, as experiments transfecting wild type Rb into the Rb negative SAOS-2 line show Rb to have a direct role in protection against radiation induced apoptosis62.

Implications for the development of cancer therapies

Recent experiments have led to the idea that apoptosis plays a pivotal role in the prevention of cancer. The genes reviewed here represent some of the best characterised examples known to modulate this process.
Mutations which affect these genes can be shown, often in synergy with other genetic lesions, to result in tumour development (see Fig. 1). However, the relationships between the acquisition of these genetic lesions and the development of cancer is complex, and highly tissue specific.

Appreciation of the tumour suppressive role played by apoptosis is provoking the design of novel cancer treatments and therapeutic strategies specifically aimed at promoting the rate of apoptosis within tumours. Perhaps the most obvious candidate for such therapy is p53. p53 null tumours do not respond well to conventional DNA damaging tumour treatments. Indeed, the inability to initiate apoptosis and eradicate resultant DNA damaged tumour cells may mean that conventional therapy will actually select for genomic instability in these tumours.

A plethora of in vitro experiments exists which strongly suggests reintroduction of p53 into null tumour cells will modulate their phenotype. Optimistically, target cells will now recognise the fact that they are malignant, presumably as a result of persistent or continuing genomic damage. Such recognition should result in either growth arrest or apoptosis of the tumour cell. At the least, one could predict that these

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![Diagram](image)

**Fig. 1** Schematic diagram illustrating how the genes referred to in this chapter can influence the balance between cell proliferation and cell death. As discussed, lesions within these genes can lead to the clonal expansion of affected cells on the basis of selective growth advantage. Where cells are characterised by impaired apoptosis there will be strong selection for further lesions in genes controlling proliferation. Conversely, in cell populations with an increased proliferative capacity, there will be strong selection for lesions conferring impaired apoptosis. As such, it is proposed that the malignant conversion of a cell does not arise from one unique genetic event, but rather from a synergy of mutations affecting both the processes of cellular proliferation and apoptosis.
Apoptosis target cells will be sensitised to conventional therapy. The prospect of clear therapeutic benefit has stimulated several different clinical trials. For example, Roth et al have used direct injection of a modified retroviral vector to deliver p53 to non-small cell tumours of the lung. This approach has produced encouraging results, with three out of a total of nine patients exhibiting tumour regression and a further three showing stabilisation of tumour growth.

There are however several reasons why the re-introduction of p53 may not offer a universally successful cancer treatment. First, one cannot assume that because a tumour lacks p53, that this will be the only lesion in the pathways mediating p53 dependant growth arrest or apoptosis. The likelihood that other lesions have occurred within these pathways will be influenced principally by the degree of usage by p53 independent mechanisms, which will determine the selective pressure for further mutation. Clearly, restoration of p53 function to null cells which have a crucial lesion in a downstream pathway will be of limited therapeutic benefit. Second, where loss of p53 gene function has arisen not from deletion, but by conformational change, re-introduction of wild-type p53 may fail to alleviate the null phenotype. Such failure may arise as a direct result of dominant negative activity by the mutant allele, or more simply by the titration of wild type p53 function.

Direct modulation of apoptosis should not be viewed as the only basis for p53 dependent therapy. One of the more encouraging strategies has arisen out of the use of a mutant adenovirus which fails to express the E1B protein. E1B normally inactivates p53, presumably in an attempt to bypass the induction of apoptosis and so permit viral replication. Without this protein, the virus can only replicate in p53 null cells, which it subsequently lyses. This differential ability to lyse p53 null cells has been used with some success to induce regression in a murine tumour model.

In summary, there is a favourable future for apoptosis based cancer therapies, whether through direct modulation of the apoptotic pathway, or from a better understanding of the apoptotic process. Ultimately, it is envisaged that these will lead to individual profiling of any given malignancy, to allow for the precise matching of therapy type. Although in its infancy, this approach may already be of some use. For example, experimental data suggest that treatment of p53 null cells with DNA damaging agents exclusively using p53 dependent pathways can actually promote further genomic instability; it is, therefore, a logical step to treat such tumours with broad spectrum agents known to activate p53-independent death.

The promise of significantly enhanced therapy engineered on the basis of apoptosis has not yet been attained. However, our understanding of the genetic control of apoptosis is becoming clearer, as is our
understanding of the relevance of apoptosis to carcinogenesis. Resolution of these two issues will inevitably lead to the application of apoptosis based strategies within the clinical setting.

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