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The role of the *p53* and *Rb-1* genes in cancer, development and apoptosis

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SUMMARY

Gene targeting using embryonal stem cells has been used to generate strains of mice with inactivating mutations at the *Rb-1* and *p53* tumour suppressor loci. Mice heterozygous for a null allele of *Rb-1* do not show retinoblastomas but instead develop pituitary tumours. Homozygotes die at between 10 and 14 days' gestation and show increased levels of both cell division and cell death by apoptosis in the haematopoietic and nervous systems. This is consistent with the view that the *Rb-1* gene product plays a general role in the maturation of precursor cells. In contrast, mice heterozygous for a null allele of *p53* are predisposed to a spectrum of tumours, while the corresponding homozygotes are viable but show a very high tumour incidence. Thymocytes from *p53* homozygotes, unlike wild-type thymocytes, do not show increased levels of apoptosis following treatment with DNA-damaging agents, while response to its induction by other agents is unaltered. Similarly, epithelial cells from the crypts of both small and large intestine of *p53*-deficient mice are resistant to the induction of apoptosis by γ-irradiation. In contrast, two other early responses of wild-type crypts to γ-irradiation, namely the G_2 block and the reduction in bromodeoxyuridine incorporation, are both largely intact in *p53*-deficient mice. These observations are consistent with the view that *p53* is responsible for monitoring DNA damage so that damaged cells can be either repaired or eliminated prior to division.

Key words: oncosuppressor gene, cancer, development, apoptosis

INTRODUCTION

An oncosuppressor gene is one whose normal function prevents the development of one or more types of cancer; such genes are also known as tumour suppressor genes or antioncogenes (reviewed by Knudson, 1993). A powerful way of investigating how such genes function is to examine the consequences of inactivating them in an experimental animal. This can be done using gene targeting in embryonal stem (ES) cells (reviewed by Hooper, 1992). These cells, established in culture from the pluripotent inner cell mass cells of the peri-implantation embryo, retain the capacity to colonise the somatic tissues and germ line of chimaeras produced by injecting them into blastocyst-stage embryos. Designed mutations can be introduced into chosen genes by introducing into the cultured cells a gene targeting DNA vector and isolating cells in which the vector has undergone homologous recombination with the endogenous gene. These mutations can then be introduced via chimaeras into the mouse germ line. I will describe the application of this approach to the *Rb-1* and *p53* oncosuppressor genes.

THE *RB-1* GENE

The paradigm for oncosuppressor genes is the retinoblastoma susceptibility gene *RB-1*. Retinoblastoma is a tumour of the retina occurring predominantly in early childhood which, although relatively uncommon, is of interest in that about 40% of all cases are familial. Knudson (1971) proposed that these tumours arise as a result of two mutational events, of which one is present in the germline in familial cases. Recent molecular analysis has shown this to be correct, and demonstrated that both events involve modification or loss of the same gene, *RB-1*. More than 90% of individuals constitutively heterozygous for an *RB-1* mutation develop retinoblastoma as a result of a somatic event occurring in one or more cells of the retina, or of its precursor tissues, that eliminates the function of the wild-type allele, usually by allele loss (reviewed by Knudson, 1993). The exact cell type of origin of retinoblastoma is the subject of some dispute, with advocates of primitive neurotubular cell, glial cell and photoreceptor cell origin, the last possibility being most easily reconcilable with biochemical, immunocytochemical and morphological properties (Rootman et al., 1987). Photoreceptor-like ultrastructural features are particularly marked in benign tumours, termed retinomas or retinocytomas, which develop in a small proportion of *RB-1* heterozygotes and are thought to result from loss of the residual wild-type allele in a more mature stage of the retinoblastoma precursor cell type (Gallie et al., 1982). These tumours have been described as cone-like (Margo et al., 1983), although this should not be over-interpreted since the organisational features that distinguish rods from cones would probably be obscured in a tumour. In addition to retinal tumours, about 15% of *RB-1* heterozygotes develop osteosarcomas. *RB-1* allele loss is also seen in sporadic lung, breast, prostate and bladder carcinomas, although no increase in incidence of these tumours is seen in germline heterozygotes (see Knudson, 1993).

The *RB-1* gene codes for a 105 kDa protein that is present in most cell types and undergoes cell cycle-dependent changes in phosphorylation as a result of the action of cyclin-dependent kinase and phosphoprotein phosphatase activity (see Hollingsworth et al., 1993). The hypophosphorylated protein associates with the cell nucleus and binds to transcription factors such as E2F, with effects that depend upon the transcription factor and in some cases also on the cell type. Transcription of genes containing an E2F recognition site is inhibited by the binding of the *RB-1* gene product; such genes include several whose products are required during S phase. It appears to depend upon the phase of the cell cycle whether E2F is predominantly associated with the *RB-1* gene product or with one of two other related proteins, p107 or p130 (Cao et al., 1992; Shirodkar et al., 1992; Li et al., 1993; Hannon et al., 1993; Cobrinik et al., 1993) and it is likely that this provides a further level at which the expression of E2F-dependent genes can be regulated. A different control element is implicated in the stimulation of *c-fos* expression by the *RB-1* gene product (Robbins et al., 1990). Further levels of complexity may be present in the form of feedback control loops, one involving *RB-1* and TGF-β1 possibly involved in the regulation of *c-myc* expression (see Hollingsworth et al., 1993) and another involving *RB-1* and a cyclin-dependent kinase inhibitor, $p16^{INK4}$ (Serrano et al., 1993). Numerous proteins other than transcription factors also associate with the *RB-1* gene product, implicating it in mechanisms unrelated to the control of gene expression. The importance of *RB-1* in cell cycle regulation is emphasised by the fact that several tumour viruses have independently evolved proteins that bind to the hypophosphorylated gene product and thus inactivate it, examples being SV40 T antigen, adenovirus E1A protein and human papillomavirus E7 protein (see Hollingsworth et al., 1993).

Rb-1 MUTANT MICE

The corresponding murine gene, designated *Rb-1,* has 91% sequence homology to the human gene and is widely expressed in mouse tissues (Bernards et al., 1989; Szekely et al., 1992). In collaboration with the laboratory of Dr Anton Berns at the Netherlands Cancer Institute, we have used gene targeting to introduce mutations into the mouse *Rb-1* gene (Clarke et al., 1992). Two other groups have also done so (Lee et al., 1992; Jacks et al., 1992). All three groups have reported similar results, and in what follows I will discuss principally our own data, with reference to those of the other groups where appropriate. We used two targeting vectors to generate the mutant *Rb-1* alleles, designed to insert, respectively, the selectable marker genes *neo* and *hyg,* in each case under the control of the *pgk-1* promoter, into exon 19 of the *Rb-1* gene (Clarke et al., 1992). Both insertions carry translation termination codons in all three reading frames and polyadenylation signals, so that in the targeted allele the *Rb-1* reading frame is truncated upstream of the coding sequences for SV40 T antigen-binding domain number 2, which is essential for the function of the gene product (Hu et al., 1990). We therefore consider both targeted alleles as null alleles.

In contrast to the situation in humans, no retinoblastomas have to date been detected in mice heterozygous for a null allele of *Rb-1.* We have considered a number of possible explanations for this. First, because of their smaller size and shorter lifespan, mice may simply have a lower probability of a somatic mutation occurring in the retina during the period when it is susceptible to tumour development, so that only a very small proportion of heterozygotes develop retinoblastomas. However, as the aggregate number of heterozygous animals examined by the three groups increases, this hypothesis becomes progressively less tenable. Second, mice may differ from humans in the level of exposure of their retinas to some required environmental agent. As laboratory mice are maintained under conditions of artificial light and are active principally in the hours of darkness, one candidate for such an environmental agent is sunlight. Intriguingly, published incidences of human retinoblastoma are significantly correlated with geographical latitude (Fig. 1) and more recent data also show this trend (M. L. Hooper and D. M. Parkin, unpublished), indicating that this hypothesis is worthy of further investiga-

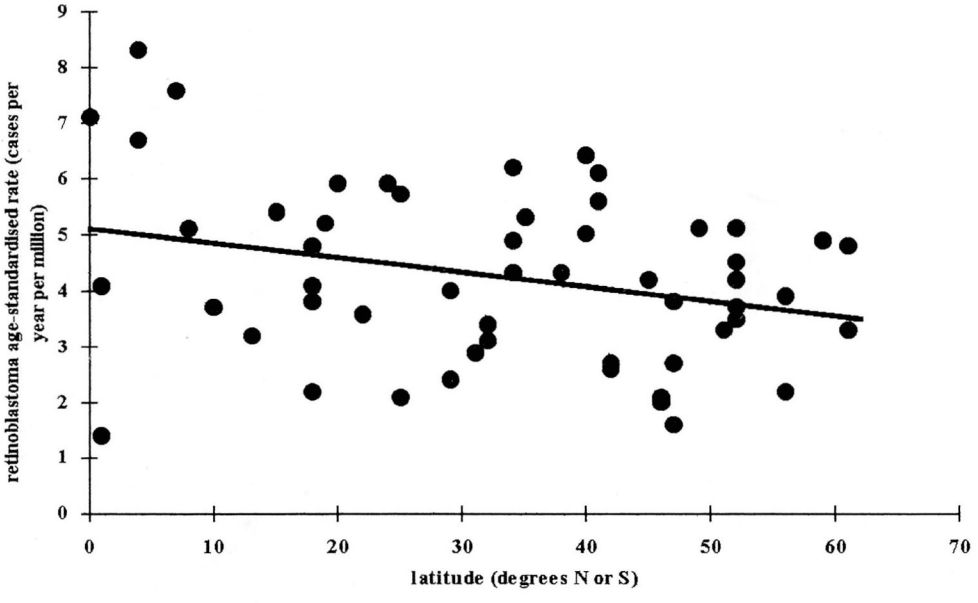

Fig. 1. Effect of geographical latitude on retinoblastoma incidence. Age-standardised rates for the first 14 years of life are taken from Parkin et al. (1988). A linear regression has been fitted to the data using the GLIM statistical package (Numerical Algorithms Group, Oxford). The slope, $-0.02606 \pm$ s.e. 0.01158, is significantly different from zero (t=2.250, P<0.05).

tion. Third, there may be a need in the mouse for an additional genetic event or events. Two observations suggest that this idea should be taken seriously: (i) retinoblastomas are uncommon if not non-existent in veterinary practice (Hogan and Albert, 1991), suggesting that humans may be unusually susceptible; (ii) it is possible to induce retinoblastoma in the mouse by expressing SV40 T antigen in the retina, thereby inactivating both the *Rb-1* and *p53* gene products (Windle et al., 1990). However, crossing into our *Rb-1* mutant stock the null allele of *p53* described below has not led to the development of any retinoblastomas (A. R. Clarke et al., unpublished observations). Fourth, in view of the relationship to photoreceptor cells discussed above, it may be significant that the photoreceptors of the predominantly nocturnal mouse are almost exclusively rods, while humans also possess cones of three different spectral sensitivities (see Bowmaker, 1991). Notwithstanding the absence of retinoblastomas in *Rb-1* heterozygous mice, pituitary adenocarcinomas develop in most if not all of these animals (Jacks et al., 1992; Hu et al., 1994; D. J. Harrison et al., unpublished observations). Hu et al. (1994) report that these tumours develop in the intermediate lobe of the pituitary, which is present only in vestigial form in adult humans. They produce α-melanocyte stimulating hormone, suggesting that they originate from corticotrope cells and, interestingly, also show patchy expression of the photoreceptor S-antigen (Hu et al., 1994).

No instance of constitutional homozygosity for a null *RB-1* allele has been reported in humans, and so the production of *Rb-1* mutant mice allowed the consequences of germline homozygosity at this locus to be studied for the first time. When heterozygous mice were intercrossed, no homozygotes survived to term, and examination of midgestation embryos revealed that homozygotes showed abnormalities in the haematopoietic and nervous systems, in both cases involving increased levels of cell death by apoptosis and overabundant or ectopic mitosis (Clarke et al., 1992; Lee et al., 1992; Jacks et al., 1992). The tissues affected correlate well with sites of high-level *Rb-1* expression (Szekely et al., 1992) and are those in which maturation of a dividing precursor cell population to a postmitotic differentiated cell occurs earliest in embryonic development, suggesting that *Rb-1* has a generalised role in the maturation of precursor cells. The presence of abnormalities in both cell division and cell death is consistent with the hypothesis that *Rb-1* functions to maintain cells in a quiescent state in which they show reduced levels of both mitosis and apoptosis (Dive and Wyllie, 1993). Interestingly, at least part of the *Rb-1*-deficient phenotype appears not to be cell-autonomous, since *Rb-1*-deficient embryonal stem cells can give rise to phenotypically normal mature B and T lymphocytes in chimaeras produced by injecting them into blastocysts homozygous for a mutation in the *Rag-2* gene (Chen et al., 1993).

THE *p53* GENE

The *p53* gene, originally discovered by virtue of its ability to form a complex with SV40 T antigen (Lane and Crawford, 1979), was at first thought to be an oncogene, but it was subsequently realised that the allele initially cloned and studied was, in fact, a mutant allele, and the wild-type gene functions as an oncosuppressor (see Knudson, 1993). Its loss is the most common allele loss in human malignancy, and germline heterozygosity for a mutant allele causes Li-Fraumeni syndrome, a predisposition to a spectrum of tumours of which breast and brain tumours are the most common (reviewed by Knudson, 1993). The gene encodes a sequence-specific DNA-binding protein that activates the expression of a reporter gene adjacent to the binding site (reviewed by Vogelstein and Kinzler, 1992). The protein is phosphorylated in a cell cycle-dependent manner, and following DNA damage its levels rise as a result of post-translational stabilisation, leading to G_1 growth arrest. It has been shown recently that the G_1 arrest is mediated by a 21 kDa cyclin-dependent kinase inhibitor, variously designated WAF-1, Cip-1 or sdi-1, whose expression is induced by p53 protein (reviewed by Nasmyth and Hunt, 1993). Loss of wild-type *p53* is associated with genomic instability, which has led to the proposal that the p53 protein functions to prevent cells entering S phase before DNA damage has been repaired (Lane, 1992). Studies on cultured cell lines have also implicated *p53* in the regulation of apoptosis (Yonish-Rouach et al., 1991; Shaw et al., 1992). Some mutant alleles of *p53* exert a dominant negative effect and are able, in the presence of wild-type *p53,* to immortalise normal early-passage cells in culture and cooperate with *ras* to induce transformation of embryo fibroblasts. This effect depends on the ability of the mutant protein to sequester wild-type protein into inactive oligomers. Such alleles lack the ability of the wild-type allele to suppress the transformation of embryo fibroblasts by other oncogenes, which is itself not dependent on ability to oligomerise (see Slingerland et al., 1993).

p53 MUTANT MICE

Mice with a targeted mutation in the *p53* gene were first reported by Donehower et al. (1992), who found that heterozygous animals developed tumours at low frequency, while homozygotes developed normally to birth but all developed tumours, of various types, in the first few months of life. Although the targeted allele carried by these mice was designed as a null allele, the possibility could not be rigorously excluded that an active polypeptide fragment could be produced from the 3′ end of the modified gene. However, we have used gene targeting to produce mice with a *p53* allele in which exons 2 to 6 are replaced by an insert that, as described above for *Rb-1,* truncates the reading frame, producing an unambiguously null allele (Clarke et al., 1993), and these mice behave similarly. Homozygous mice develop tumours, predominantly T cell lymphomas, during the first six months of life; in heterozygotes, which develop tumours later, soft tissue sarcomas are relatively more important (Purdie et al., 1994). Some mice developed pathological signs without obvious tumour development, and most of these showed reactive lymphoid hyperplasia, suggesting a perturbation of the immune system. A similar tumour spectrum has been reported in a third *p53* mutant strain (Jacks et al., 1994). Small differences in tumour spectrum between strains are probably a consequence of differences in genetic background (Harvey et al., 1993a). Most tumours in our *p53* heterozygotes had suffered loss of the wild-type *p53* allele, indicating that the gene behaves as a classical oncosuppressor. In contrast to the pituitary tumours

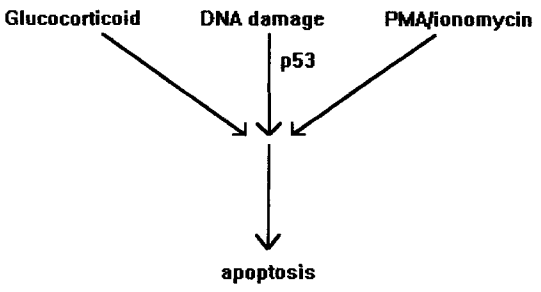

Fig. 2. Pathways to apoptosis in thymocytes. PMA (phorbol myristoyl 13-acetate) and ionomycin provide a stimulus that mimics the effect of crosslinking the T-cell antigen receptor, thereby modelling the physiological process of negative selection in the thymus (see Clarke et al., 1993).

occurring in the *Rb-1* heterozygotes, most of the tumours in *p53* heterozygous and homozygous mice contained an aneuploid or polyploid cell population as assessed by flow cytometry, consistent with the concept that, in the absence of functional *p53,* cells enter division without having repaired DNA damage, and this leads to abnormalities in chromosome segregation (Lane, 1992).

An important clue to the mechanism underlying the tumour suppressor activity of *p53* was provided by studies of thymocyte apoptosis. Wild-type thymocytes placed in culture in serum-free medium spontaneously undergo apoptosis over a 1- to 2-day period, and the rate of this apoptosis can be stimulated by a variety of stimuli including physiological agents such as glucocorticoids and DNA-damaging agents such as γ-radiation. Thymocytes from *p53* homozygous mice showed normal response to agents of the former class, but were resistant to induction of apoptosis by agents of the second class (Clarke et al., 1993; Lowe et al., 1993b). Heterozygous cells showed an intermediate response. This suggested that stimuli can feed into the apoptosis pathway via different routes, and that *p53* is required in a branch route linking DNA damage to the main apoptosis pathway (Fig. 2).

In order to investigate whether this behaviour is peculiar to thymocytes, we have also examined the response of epithelial cells of intestinal crypts to γ-irradiation in vivo. Four hours after irradiation, crypt cells from both small intestine and colon of wild-type mice showed abundant apoptosis, but corresponding cells from *p53* homozygotes were completely resistant to induction of apoptosis. Again, cells from heterozygotes showed an intermediate response. In contrast, two other early responses of wild-type crypts to γ-irradiation, namely reduction in the proportion of cells in S phase, as assessed by the bromodeoxyuridine labelling index, and growth arrest in the G_2 phase of the cell cycle (reviewed by Potten, 1990) were both largely intact in *p53*-deficient animals (Clarke et al., 1994). Merritt et al. (1994) also observed resistance of intestinal epithelial cells from *p53* homozygous mutant mice to induction of apoptosis by γ-irradiation, although they did not observe a reduction in the response of cells from heterozygotes, perhaps because they used a higher irradiation dose. In both studies the low background level of apoptosis in the intestine appeared to be *p53*-independent, indicating that, as with thymocytes, *p53* is required to link apoptosis to DNA damage rather than being required for apoptosis per se. This is consistent with the observation that the development of homozygous *p53*-deficient mice to birth is normal, implying no anomalies in developmental programmed cell death (Donehower et al., 1992), and with the finding that *p53* is not required for the apoptosis that occurs in prostatic glandular cells following androgen ablation (Berges et al., 1993).

An important question that remains is that of how cells make a decision between different *p53*-mediated responses to DNA damage, i.e. a decision between entry into growth arrest, thereby possibly allowing DNA repair, and entry into apoptosis. Relevant to this question is recent work of Lowe et al. (1993a) who showed, using fibroblasts of different *p53* genotypes, that the apoptotic response in these cells is seen only when G_1 growth arrest is bypassed by expressing the adenovirus E1A protein in the cells, suggesting that in general the choice of response depends upon what other genes the cell is expressing. Fibroblasts from *p53* mutant mice have also been used to confirm that p53-deficiency promotes genetic instability and to show that it facilitates immortalisation (Harvey et al., 1993b). Enhanced proliferative potential has been noted in a variety of cell types from *p53*-deficient mice (Tsukuda et al., 1993).

It seems reasonable to conclude that the reason that *p53*-deficient mice develop tumours is that damaged, potentially tumourigenic cells fail to be eliminated by the normal apoptotic pathway and instead grow into tumours. This emphasises the fact that the regulation of cell death can be as important as the regulation of cell division in determining whether tumours develop. It also has implications for cancer therapy in that some anti-cancer drugs appear to exert their cytotoxic effect by induction of the endogenous apoptotic pathway (reviewed by Dive and Wyllie, 1993) and therefore loss of *p53*, the most common allele loss in human cancer, may be an important mechanism of resistance to such drugs. The advent of novel drugs specifically designed to activate the natural suicide pathway of the cell is likely to provide valuable therapeutic agents for the treatment of cancer in future years.

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REFERENCES

Berges, R. R., Furuya, Y., Remington, L., English, H. F., Jacks, T. and Isaacs, J. T. (1993). Cell proliferation, DNA repair, and p53 function are not required for programmed death of prostatic glandular cells induced by androgen ablation. *Proc. Nat. Acad. Sci. USA* **90**, 8910-8914.

Bernards, R., Schackleford, G. M., Gerber, M. R., Horowitz, J. M., Friend, S. H., Schartl, M., Bogenmann, E., Rapaport, J. M., McGee, T., Dryja, T. P. and Weinberg, R. A. (1989). Structure and expression of the murine retinoblastoma gene and characterization of its encoded protein. *Proc. Nat. Acad. Sci. USA* **86**, 6474-6478.

Bowmaker, J. K. (1991). The evolution of vertebrate visual pigments and photoreceptors. In *Vision and Visual Dysfunction*, vol. 2 (ed. J. R. Cronly-Dillon and R. L. Gregory) pp. 63-81. London: Macmillan Press.

Cao, L., Faha, B., Dembski, M., Tsai, L.-H., Harlow, E. and Dyson, N. (1992). Independent binding of the retinoblastoma protein and p107 to the transcription factor E2F. *Nature* **355**, 176-179.

Chen, J., Gorman, J. R., Stewart, V., Williams, B., Jacks, T. and Alt, F. W. (1993). Generation of normal lymphocyte populations by Rb-deficient embryonic stem cells. *Curr. Biol.* **3**, 405-413.

Clarke, A. R., Robanus Maandag, E., van Roon, M., van der Lugt, N. M. T., van der Valk, M., Hooper, M. L., Berns, A. and te Riele, H. (1992). Requirement for a functional *Rb-1* gene in murine development. *Nature* **359**, 328-330.

Clarke, A. R., Purdie, C. A., Harrison, D. J., Morris, R. G., Bird, C. C., Hooper, M. L. and Wyllie, A. H. (1993). Thymocyte apoptosis induced by p53-dependent and independent pathways. *Nature* **362**, 849-852.

Clarke, A. R., Gledhill, S., Hooper, M. L., Bird, C. C. and Wyllie, A. H. (1994). p53-dependence of early apoptotic and proliferative responses within the mouse intestinal epithelium following γ-irradiation. *Oncogene* **9**, 1767-1773.

Cobrinik, D., Whyte, P., Peeper, D. S., Jacks, T. and Weinberg, R. A. (1993). Cell cycle-specific association of E2F with the p130 E1A-binding protein. *Genes Dev.* **7**, 2392-2404.

Dive, C. and Wyllie, A. H. (1993). Apoptosis and cancer chemotherapy. In *Frontiers in Pharmacology: Cancer Chemotherapy* (ed. J. A. Hickman and T. T. Tritton), pp 21-56. Oxford: Blackwell Scientific.

Donehower, L. A., Harvey, M., Slagle, B. M., McArthur, M. J., Montgomery, C. A. Jr, Butel, J. S. and Bradley, A. (1992). Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature* **356**, 215-221.

Gallie, B. L., Ellsworth, R. M., Abramson, D. H. and Phillips, R. A. (1982). Retinoma: spontaneous regression of retinoblastoma or benign manifestation of the mutation? *Brit. J. Cancer* **45**, 513-521.

Hannon, G. J., Demetrick, D. and Beach, D. (1993). Isolation of the Rb-related p130 through its interaction with CDK2 and cyclins. *Genes Dev.* **7**, 2378-2391.

Harvey, M., McArthur, M. J., Montgomery, C. A. Jr, Bradley, A. and Donehower, L. A. (1993a). Genetic background alters the spectrum of tumors that develop in p53-deficient mice. *FASEB J.* **7**, 938-943.

Harvey, M., Sands, A. T., Weiss, R. S., Hegi, M. E., Wiseman, R. W., Pantazis, P., Giovanella, B. C., Tainsky, M. A., Bradley, A. and Donehower, L. A. (1993b). *In vitro* growth characteristics of embryo fibroblasts isolated from p53-deficient mice. *Oncogene* **8**, 2457-2467.

Hogan, R. N. and Albert, D. M. (1991). Does retinoblastoma occur in animals? *Prog. Vet. Comp. Ophthalmol.* **1**, 73-82.

Hollingsworth, R. E. Jr, Hensey, C. E. and Lee, W.-H. (1993). Retinoblastoma protein and the cell cycle. *Curr. Opin. Genet. Dev.* **3**, 55-62.

Hooper, M. L. (1992). *Embryonal Stem Cells: Introducing Planned Changes into the Animal Germline*. Harwood Academic Publishers gmbh, Chur, Switzerland.

Hu, N., Gutsmann, A., Herbert, D. C., Bradley, A., Lee, W.-H. and Lee, E. Y.-H. P. (1994). Heterozygous *Rb-1*$^{\Delta 20}$/+ mice are predisposed to tumours of the pituitary gland with a nearly complete penetrance. *Oncogene* **9**, 1021-1027.

Hu, Q., Dyson, N. and Harlow, E. (1990). The regions of the retinoblastoma protein needed for binding to adenovirus E1A or SV40 large T antigen are common sites for mutations. *EMBO J.* **9**, 1147-1155.

Jacks, T., Fazeli, A., Schmitt, E. M., Bronson, R. T., Goodell, M. A. and Weinberg, R. A. (1992). Effects of an Rb mutation in the mouse. *Nature* **359**, 295-300.

Jacks, T., Remington, L., Williams, B. O., Schmitt, E. M., Halachmi, S., Bronson, R. T. and Weinberg, R. A. (1994). Tumour spectrum analysis in p53 mutant mice. *Curr. Biol.* **4**, 1-7.

Knudson, A. G. (1971). Mutation and cancer: statistical study of retinoblastoma. *Proc. Nat. Acad. Sci. USA* **68**, 820-823.

Knudson, A. G. (1993). Antioncogenes and human cancer. *Proc. Nat. Acad. Sci. USA* **90**, 10914-10921.

Lane, D. P. and Crawford, L. V. (1979). T antigen is bound to a host protein in SV40-transformed cells. *Nature* **278**, 261-263.

Lane, D. P. (1992). Cancer: p53, guardian of the genome. *Nature* **358**, 15-16.

Lee, E. Y.-H. P., Chang, C.-Y., Hu, N., Wang, Y.-C. J., Lai, C.-C., Herrup, K., Lee, W.-H. and Bradley, A. (1992). Mice deficient for Rb are nonviable and show defects in neurogenesis and haematopoiesis. *Nature* **359**, 288-294.

Li, Y., Graham, C., Lacy, S., Duncan, A. M. V. and Whyte, P. (1993). The adenovirus E1A-associated 130-kD protein is encoded by a member of the retinoblastoma gene family and physically interacts with cyclins A and E. *Genes Dev.* **7**, 2366-2377.

Lowe, S. W., Ruley, H. E., Jacks, T. and Housman, D. E. (1993a). p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. *Cell* **74**, 957-967.

Lowe, S. W., Schmitt, E. M., Smith, S. W., Osborne, B. A. and Jacks, T. (1993b). p53 is required for radiation-induced apoptosis in mouse thymocytes. *Nature* **362**, 847-849.

Margo, C., Hidayat, A., Kopelman, J. and Zimmerman, L. E. (1983). Retinocytoma: a benign variant of retinoblastoma. *Arch. Ophthalmol.* **101**, 1519-1531.

Merritt, A. J., Potten, C. S., Kemp, C. J., Hickman, J. A., Balmain, A., Lane, D. P. and Hall, P. A. (1994). The role of p53 in spontaneous and radiation-induced apoptosis in the gastrointestinal tract of normal and p53-deficient mice. *Cancer Res.* **54**, 614-617.

Nasmyth, K. and Hunt, T. (1993). Cell cycle: Dams and sluices. *Nature* **366**, 634-635.

Parkin, D. M., Stiller, C. A., Bieber, A., Draper, G. J., Terracini, B. and Young, J. L. (1988). International incidence of childhood cancer. *Int. Agency Res. Cancer Sci. Publ.* **87**.

Potten, C. S. (1990). A comprehensive study of the radiobiological response of the murine (BDF1) small intestine. *Int. J. Radiat. Biol.* **58**, 925-973.

Purdie, C. A., Harrison, D. J., Peter, A., Dobbie, L., White, S., Howie, S. E. M., Salter, D. M., Bird, C. C., Wyllie, A. H., Hooper, M. L. and Clarke, A. R. (1994). Tumour incidence, spectrum and ploidy in mice with a large deletion in the p53 gene. *Oncogene* **9**, 603-609.

Robbins, P. D., Horowitz, J. M. and Mulligan, R. C. (1990). Negative regulation of human *c-fos* expression by the retinoblastoma gene product. *Nature* **346**, 668-671.

Rootman, J., Carruthers, J. D. A. and Miller, R. R. (1987). Retinoblastoma. *Perspect. Pediatr. Pathol.* **10**, 208-258.

Serrano, M., Hannon, G. J. and Beach, D. (1993). A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. *Nature* **366**, 704-707.

Shaw, P., Bovey, R., Tardy, S., Sahli, R., Sordat, B. and Costa, J. (1992). Induction of apoptosis by wild-type p53 in a human colon tumour-derived cell line. *Proc. Nat. Acad. Sci. USA* **89**, 4495-4499.

Shirodkar, S., Ewen, M., DeCaprio, J. A., Morgan, J., Livingston, D. M. and Chittenden, T. (1992). The transcription factor E2F interacts with the retinoblastoma product and a p107-cyclin A complex in a cell cycle-regulated manner. *Cell* **68**, 157-166.

Slingerland, J. M., Jenkins, J. R. and Benchimol, S. (1993). The transforming and suppressor functions of p53 alleles: effects of mutations that disrupt phosphorylation, oligomerization and nuclear translocation. *EMBO J.* **12**, 1029-1037.

Szekely, L., Jiang, W.-Q., Bulic-Jakus, F., Rosen, A., Ringertz, N., Klein, G. and Wiman, K.G. (1992). Cell type and differentiation dependent heterogeneity in retinoblastoma protein expression in SCID mouse fetuses. *Cell Growth Differ.* **3**, 149-156.

Tsukuda, T., Tomooka, Y., Takai, S., Ueda, Y., Nishikawa, S., Yagi, T., Tokunaga, T., Takeda, N., Suda, Y., Abe, S., Matsuo, I., Ikawa, Y. and Aizawa, S. (1993). Enhanced proliferative potential in culture of cells from p53-deficient mice. *Oncogene* **8**, 3313-3322.

Vogelstein, B. and Kinzler, K. W. (1992). p53 Function and dysfunction. *Cell* **70**, 523-526.

Windle, J. J., Albert, D. M., O'Brien, J. M., Marcus, D. M., Disteche, C. M., Bernards, R. and Mellon, P. L. (1990). Retinoblastoma in transgenic mice. *Nature* **343**, 665-669.

Yonish-Rouach, E., Resnitzky, D., Lotem, J., Sachs, L., Kimchi, A. and Oren, M. (1991). Wild-type p53 induces apoptosis of myeloid leukaemic cells that is inhibited by interleukin-6. *Nature* **352**, 345-347.