MUTANT MICE SHED NEW LIGHT ON RETINOBLASTOMA

M.L. Hooper[*]

Retinoblastomas are childhood retinal tumours of which about 40% of all cases are familial. Knudson[1] proposed that these tumours arise as a result of two mutational events, and that in familial cases one mutation is inherited in the germline (constitutional) and one is acquired somatically, while sporadic cases result from two somatic events. Recent molecular analysis has confirmed that the latter is true, and demonstrated that both events involve modification or loss of an allele of the same gene, *RB-1*.

THE *RB-1* GENE

The *RB-1* gene has become the paradigm for a class of genes termed 'tumour suppressor genes', whose defining feature is that when functioning normally they prevent the development of one or more types of cancer.[2] More than 90% of individuals constitutionally heterozygous for an *RB-1* mutation develop retinoblastoma as a result of the occurrence of a somatic event in one or more cells of the retina (or of its precursor tissues) that eliminates the function of the wild-type allele (i.e. the normal copy of the gene), most commonly by allele loss (reviewed in reference 3). There are several candidates for the exact cell type of origin of retinoblastoma including primitive neurotubular cells, glial cells and photoreceptor cells, the last possibility being most consistent with biochemical, immunocytochemical and morphological properties of the tumour.[4] 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 cell from the same lineage as the retinoblastoma precursor cell type but at a more mature stage of development.[5] These tumours have been described as cone-like,[6] although this should not be over-interpreted since the structural features that distinguish rods from cones in retinal epithelium would probably be obscured in a tumour. In addition to retinal tumours, about 15% of *RB-1* heterozygotes develop osteosarcomas.[3] *RB-1* allele loss also occurs sporadically in carcinomas of the breast, prostate, lung and bladder, although there is no detectable increase in incidence of these tumours in germline heterozygotes.[3]

FUNCTION OF THE *RB-1* GENE

The function of the protein coded by the *RB-1* gene is now quite well understood at the biochemical level: it is present in most cell types and forms part of a pathway involved in the regulation of the cell cycle. Two other, structurally related, proteins, coded by distinct genes, *p107* and *p130,* have functions similar but not identical to that of *RB-1.* This is not discussed in detail, but the interested reader is referred to a recent review by Mulligan and Jacks.[7] The biochemical analysis, however, sheds relatively little light on the question of why inactivation of the gene leads specifically to retinoblastoma and not to tumours in other tissues.

A powerful means of investigating the role of a gene in the function of the whole body is to examine the consequences of inactivating it in an experimental animal. As recently as ten years ago, animals with mutations in a chosen gene could only be obtained by chance, but a group of techniques, known collectively as 'gene targeting', has recently been developed in the mouse which makes it possible to introduce designed mutations into chosen genes. The technical details were reviewed recently elsewhere.[8] There are, of course, many differences between mice and humans, and it is therefore not surprising that the effects of similar mutations in the two species can show both parallels and contrasts. It has sometimes been argued that this makes the study of mice irrelevent to an understanding of human disease. However, this is far from the case: even where the effects are different, comparison of the two species can generate hypotheses whose relevance to human pathology can be directly tested. Here I will review how this has been possible in the case of retinoblastoma.

PHENOTYPE OF MOUSE *Rb-1* MUTANTS

The mouse gene, designated *Rb-1,* has 91% sequence homology to the human *RB-1* gene,[9] and is clearly the equivalent gene. Unlike humans of similar genotype, mice heterozygous for a *Rb-1* null mutation (i.e. a mutation that inactivates the gene) do not develop retinoblastomas but do develop pituitary adenocarcinomas.[10-13] The latter tumours develop from the melanotroph cells in the intermediate lobe of the hypophysis, a structure which is present only in vestigial form in the human adult; this probably accounts for the absence of corresponding tumours in human heterozygotes. In addition to the pituitary tumours, medullary carcinoma of the thyroid gland develops in some, but not all, of the sublines of *Rb-1* heterozygous mice that have been independently produced in different laboratories.[12,13] The difference between the sublines may be a consequence of differences in the targeted *Rb-1* allele, in the genetic background or in environmental exposure.

No case of constitutional homozygosity for a null *RB-1* allele has ever been documented in humans, and so it was only when *Rb-1* mutant mice were produced that it became possible for the first time to study the consequences of germline homozygosity at this locus. When heterozygous mice were intercrossed, homozygous embryos failed to survive to term, and at mid-gestation showed abnormalities in the haematopoietic and nervous systems, which in both systems involved increased levels of apoptotic cell death

[*]*Professor of Molecular Pathology,*
Sir Alastair Currie Cancer Research Campaign Laboratories, Department of Pathology, Molecular Medicine Centre, University of Edinburgh, Western General Hospital, Crewe Road, Edinburgh EH4 2XU

and overabundant or ectopic mitosis (reviewed in reference 14). The tissues affected normally show high-level *Rb-1* expression and are those in which a mitotically-active precursor cell population undergoes maturation earliest in embryonic development to a postmitotic differentiated cell. This suggests that *Rb-1* has a generic role in the maturation of precursor cells. The presence of parallel effects on cell division and cell death is consistent with the hypothesis that *Rb-1* functions to maintain cells in a quiescent state characterised by reduced levels of both mitosis and apoptosis.[15]

Effects of homozygosity on events occurring later in development have been studied in chimaeras: mice consisting of a patchwork of cells of different genotype, in this case *Rb-1*-null and wild-type.[16,17] The presence of the normal cells allowed survival of the chimaeras beyond the stage where entirely *Rb-1*-null embryos die and, indeed, to even beyond birth. The *Rb-1*-null cells contributed, albeit in some instances at reduced levels, to all adult tissues examined. No retinoblastomas were seen in these chimaeras, while pituitary tumours similar to those seen in heterozygotes developed from the *Rb-1*-null cells, but with an earlier time of onset consistent with the lack of requirement for somatic allele loss.

A ROLE FOR SUNLIGHT IN RETINOBLASTOMA FORMATION?

It is interesting to consider why no retinoblastomas have yet been detected in *Rb-1* heterozygous mice. There are 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 susceptible period, so that only a very small proportion of heterozygotes develop retinoblastomas. However, as the aggregate number of heterozygous animals examined increases without any retinoblastomas being found, this hypothesis becomes progressively less tenable as the sole explanation. Second, in view of their possible derivation from photoreceptor cells discussed above, it may be significant that the retina of the predominantly nocturnal mouse has almost exclusively rod photoreceptors, while humans also possess cones of three different spectral sensitivities.[18] Third, mice may differ from humans in the level of exposure of their retinas to some environmental agent required for retinoblastoma to develop. 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. This hypothesis stimulated an analysis of the incidence of retinoblastoma in human populations at different geographical locations, which revealed that, as predicted by the hypothesis, retinoblastoma incidence increases significantly with ambient erythemal dose of ultraviolet B radiation (UVB) from sunlight.[19] Such an effect had not previously been detected, perhaps because it is less severe than that reported for other tumours such as squamous cell carcinoma of the eye.[20]

A consideration of this leads to a number of possible reasons for this effect. First, it could be a consequence of ascertainment bias, due to variations either in under- or over-reporting of retinoblastoma, or in estimating the size of the population from which the patients are drawn. Second, it could be due to genetic differences between the populations under study in the form of differences in frequency of germline *RB-1* mutations, or differences in frequency of alleles at other loci that influence the frequency of somatic events leading to loss of heterozygosity at the *RB-1* locus; either of which might result, for instance, from variations in the racial composition of populations. Third, it could be a direct effect of exposure to UVB, other wavelengths present in sunlight, or another environmental variable correlated with sunlight exposure. The most obvious hypothesis is that it is due to an effect of sunlight on somatic events leading to *RB-1* inactivation. Based on this hypothesis, since individuals inheriting a germline *RB-1* mutation almost invariably develop bilateral retinoblastomas, one would predict that the effect would be due largely or entirely to unilateral tumours, which in general occur sporadically and require two somatic events. This is indeed true (Figure 1).

This argues against the hypothesis advanced above that the association of retinoblastoma incidence with ambient UVB dose is due to ascertainment bias, which would be expected to affect data on unilateral and bilateral tumours in a similar fashion. It also argues that it is not due to differences in *RB-1* germline mutation frequency, which would affect bilateral tumour incidence preferentially. One cannot formally exclude the hypotheses that it is due either to differences in allele frequencies at other loci, or to an environmental variable correlated with sunlight exposure, but a direct effect of sunlight provides the most ready explanation of the data. This would be consistent with the mutation spectrum of sporadic retinoblastoma (reviewed in reference 21): first-hit lesions that convert normal cells to heterozygous mutant cells include some transitions in which one pyrimidine base, cytosine, is replaced by the other, thymine, consistent with action of UV radiation. However, instances in which two adjacent cytosine residues are both converted to thymine (CC to TT), a type of change that is more diagnostic of UV mutagenesis, have not been reported. Second-hit lesions that inactivate the remaining normal gene copy usually involve gross chromosomal change and are therefore less informative. To allow this hypothesis to be tested further it would be helpful for more centres to report the laterality of tumours.

Notwithstanding this effect, differences in exposure to ultraviolet radiation between mice and humans are not, on their own, sufficient to account for the lack of retinoblastomas in heterozygous *Rb-1* mutant mice, since controlled exposure of the mice to fluorescent light with a daylight spectrum has not led to the development of any retinoblastomas (J.F. Armstrong, M.H. Kaufman and M.L. Hooper, unpublished observations). This leaves open the possibility that it is important in combination with other differences. The latter could include a requirement in the mouse for an additional genetic event or events, and indeed it has recently been reported[22] that retinoblastomas developed in six of 14 chimaeric eyes in mice containing cells doubly homozygous for mutant alleles of *Rb-1* and *p107*. This demonstrates that, in addition to *Rb-1* inactivation, retinoblastoma formation in the mouse requires mutation of *p107*, and probably yet a further event involving an unidentified gene that occurred somatically in the chimaeras. This may reflect differences in gene expression in the target cell population between mouse and human, although there is not at present a ready explanation for such differences.

Nonetheless, whatever the complete explanation for the difference in retinoblastoma incidence between mouse and human heterozygotes, it has stimulated an analysis that

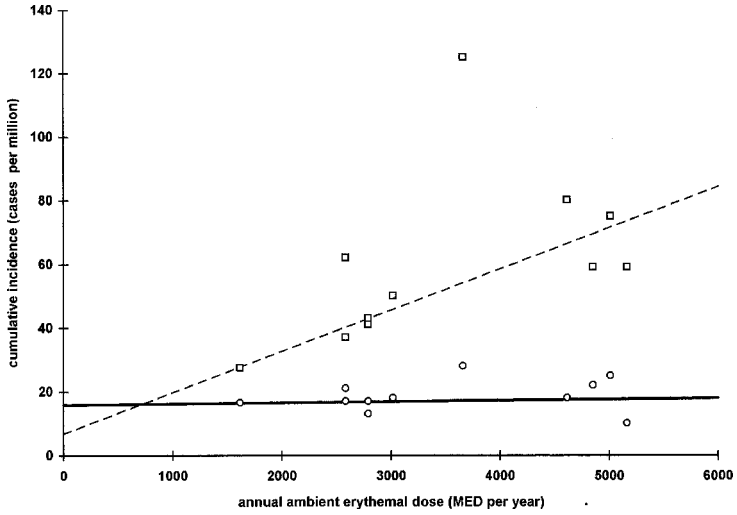

FIGURE 1
Cumulative incidence of unilateral (squares) and bilateral (circles) retinoblastoma for children of ages 0-14 at different geographical locations, plotted as a function of annual ambient erythemal dose of UVB. Regression lines have been fitted separately to unilateral data - broken line, slope 0.01295 ± s.e 0.001958, significantly different from 0 (t_9 = 6.614, $P < 0.001$) - and to bilateral data - bold continuous line, slope 0.0003361 ± s.e 0.0008433, not significantly different from zero (t_9 = 0.3986, $P > 0.5$). From reference 19, © Cancer Research Campaign 1999. Reproduced with the permission of the *British Journal of Cancer* and the Cancer Research Campaign.

has revealed a previously unsuspected association between UVB exposure and the incidence of unilateral, but not bilateral, retinoblastoma in human populations. These studies illustrate how differences between human and mouse phenotypes can generate testable hypotheses that lead to an increase in knowledge about human disease.

REFERENCES

1 Knudson AG. Mutation and cancer: statistical study of retinoblastoma. *Proc Nat Acad Sci USA* 1971; **68:**820-3.

2 Kinzler KW, Vogelstein B. Familial cancer syndromes: the role of caretakers and gatekeepers. In: Vogelstein B, Kinzler KW (eds), *The genetic basis of human cancer* New York: McGraw-Hill, 1998; 241-2.

3 Knudson AG. Antioncogenes and human cancer. *Proc Nat Acad Sci USA* 1993; **90:**10914-21.

4 Rootman J, Carruthers JDA, Miller RR. Retinoblastoma. *Perspect Pediatr Pathol* 1987; **10:**208-58.

5 Gallie BL, Ellsworth RM, Abramson DH, Phillips RA. Retinoma: spontaneous regression of retinoblastoma or benign manifestation of the mutation? *Brit J Cancer* 1982; **45:**513-21.

6 Margo C, Hidayat A, Kopelman J, Zimmerman LE. Retinocytoma: a benign variant of retinoblastoma. *Arch Ophthalmol* 1983; **101:**1519-31.

7 Mulligan G, Jacks T. The retinoblastoma gene family: cousins with overlapping interests. *Trends Genet* 1998; **14:**223-9.

8 Hooper ML. Tumour suppressor gene mutations in humans and mice: parallels and contrasts. *EMBO J* 1998; **17:**6783-9.

9 Bernards R, Schackleford GM, Gerber MR *et al.* Structure and expression of the murine retinoblastoma gene and characterization of its encoded protein. *Proc Nat Acad Sci USA* 1989; **86:**6474-8.

10 Jacks T, Fazeli A, Schmitt EM *et al.* Effects of an Rb mutation in the mouse. *Nature* 1992; **359:**295-300.

11 Hu N, Gutsmann A, Herbert DC *et al.* Heterozygous *Rb-1*$^{\Delta 20}$/+ mice are predisposed to tumors of the pituitary gland with a nearly complete penetrance. *Oncogene* 1994; **9:**1021-7.

12 Williams BO, Remington L, Albert DM *et al.* Cooperative tumorigenic effects of germline mutations in *Rb* and *p53*. *Nat Genet* 1994; **7:**480-4.

13 Harrison DJ, Hooper ML, Armstrong JF, Clarke AR. Effects of heterozygosity for the *Rb-1*^{t19neo} allele in the mouse. *Oncogene* 1995; **10:**1615-20.

14 Hooper ML. Analysis of oncosuppressor gene function in the mouse by gene targeting. In: Zander A, Ostertag W, Afanasiev B, Grosveld F. (eds). *Gene technology in analysis and treatment of malignant and inherited diseases related to development: NATO ASI Series, Subseries H 'Cell Biology' vol. 94* Berlin: Springer-Verlag, 1996; 263-81.

15 Bellamy COC, Malcomson RDG, Harrison DJ, Wyllie AH. Cell death in health and disease: the biology and regulation of apoptosis. *Semin Cancer Biol* 1995; **6:**3-16.

16 Williams BO, Schmitt EM, Remington L *et al.* Extensive contribution of Rb-deficient cells to adult chimeric mice with limited histopathological consequences. *EMBO J* 1994; **13:**4251-9.

17 Robanus-Maandag EC, van der Valk M, Vlaar M *et al.* Developmental rescue of an embryonic-lethal mutation in the retinoblastoma gene in chimeric mice. *EMBO J* 1994; **13:**4260-8.

18 Bowmaker JK. The evolution of vertebrate visual pigments and photoreceptors. In: Cronly-Dillon JR, Gregory RL (eds) *Vision and visual dysfunction vol. 2* London: Macmillan Press, 1991; 63-81.

19 Hooper ML. Is sunlight an aetiological agent in the genesis of retinoblastoma? *Brit J Cancer* 1999; **79:**1273-6.

20 Newton R, Ferlay J, Reeves G *et al.* Effect of ambient solar ultraviolet radiation on incidence of squamous-cell carcinoma of the eye. *Lancet* 1996; **347:**1450-1.

21 Murphree AL, Munier FL. Retinoblastoma. In: Ryan SJ (editor in-chief). *Retina, 2nd ed* St. Louis: Mosby, 1994; 571-86.

22 Robanus Maandag E, Dekker M, van der Valk M *et al.* p107 is a suppressor of retinoblastoma development in pRb-deficient mice. *Genes Dev* 1998; **12:**1599-609.