EMBO MEMBER'S REVIEW

Tumour suppressor gene mutations in humans and mice: parallels and contrasts

Martin L.Hooper

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

e-mail: m.hooper@ed.ac.uk

Tumour suppressor genes prevent cancer development. They can be identified by studying humans, but a full understanding of the mechanisms of their action requires the production of animal models. Mice with mutations in tumour suppressor genes can be produced by gene targeting. The phenotypic consequences of tumour suppressor gene mutations in mice and humans show parallels and contrasts, and both can contribute to the elucidation of disease processes.

Keywords: cancer genetics/Denys–Drash syndrome/gene targeting/retinoblastoma/tumour suppressor gene

Introduction

A tumour suppressor gene is one which, when functioning normally, prevents the development of one or more types of cancer (reviewed by Knudson, 1993). Loss of function of both alleles is required for tumorigenesis. In some cases this results from two somatic mutational events, while in others a mutation in one allele is inherited in the germline and the other occurs somatically. Individuals heterozygous for such a germline mutation are at increased risk of developing tumours because of the high probability of a somatic mutation occurring in the remaining normal allele in at least one cell in a susceptible tissue, and this results in the existence of a number of human familial cancer syndromes (Table I). Kinzler and Vogelstein have subdivided tumour suppressor genes into gatekeepers and caretakers (see Kinzler and Vogelstein, 1998), the former being genes that directly regulate tumour growth by inhibiting cell proliferation or promoting death, while the latter are genes whose inactivation causes genetic instability which in turn leads to mutations that promote tumour growth. Some caretaker genes exert an effect only if both alleles are inherited through the germline in mutant form; however, for reasons of space, I have included in Table I only genes for which human heterozygotes have a cancer-prone phenotype.

While tumour suppressor genes can be identified by studying humans, a full understanding of the mechanisms of their action requires the production of animal models. Mice are most commonly chosen because it is possible to introduce designed mutations into chosen genes by gene targeting (reviewed in Hooper, 1992). A specially designed targeting vector containing segments of homology to the chosen gene is introduced by electroporation into

embryonal stem (ES) cells in culture. Homologous recombination between the vector and one chromosomal copy of the gene produces heterozygous (+/–) ES cells. These, on injection into blastocyst-stage embryos, give rise to +/– ↔ +/+ chimeric mice (Figure 1). These can be bred to produce germline heterozygotes which can then be crossed to study the effects of homozygosity.

Many mutations cause embryonic lethality when homozygous, but it is often possible to achieve development of homozygous cells to a later stage in chimeras with wild-type cells. Such –/– ↔ +/+ chimeras can be produced from ES cells in which both alleles are mutant. The latter can be produced by either of two routes (Figure 1). One is to carry out a second round of gene targeting on heterozygous cells to modify the remaining wild-type gene. The other is to subject heterozygous cells in which the mutant allele carries a *neo* gene, derived from the targeting vector, to selection in a high concentration of geneticin to enrich for cells carrying two copies of *neo* (Mortensen *et al.*, 1992). The second method yields cells in which the remaining wild-type allele is replaced by a copy of the targeted allele. The mechanism by which this occurs has not been studied, but it is assumed to involve mitotic recombination. If this is the case, the exchanged segment is likely to involve not only the target locus but substantial lengths of flanking sequence, and this may introduce unwanted changes into the genome if there is a significant linked locus that is subject to imprinting: the homozygous cells may carry two maternal or two paternal copies of the linked locus. In such a situation, as is the case with *Rb-1* (see later), the sequential targeting approach is to be preferred.

Another recently developed approach to circumventing some of the limitations of this technology is conditional gene targeting (Figure 2), which allows the production of a chosen mutation in a chosen tissue and/or at a chosen stage of development. This depends on a site-specific recombinase such as cre, which catalyses recombination between short substrate sequences, *loxP*, that do not occur naturally in the mammalian genome. It is possible by gene targeting to introduce *loxP* sequences into introns of a gene in such a way that normal function is preserved: this operation is termed ‘floxed’. Mice carrying floxed alleles show no phenotypic alteration, but introduction of a transgene that expresses cre in the required spatial and/or temporal manner leads to deletion of the chromosomal segment between the *loxP* sequences in the cre-expressing cells, producing the desired functional change (Porter, 1998).

In the case of the genes listed in Table I, it has not been possible to study human homozygotes because of their extreme rarity, so study of the mouse homozygote phenotype is a very valuable way of obtaining information about the normal function of these genes. With the

Table I. Some tumour suppressor genes and their mutant phenotypes in human and mouse

Gene	Human heterozygote phenotype	Mouse heterozygote phenotype	Mouse homozygote phenotype
<i>RB-1</i>	Retinoblastoma, osteosarcoma ^a	Pituitary adenocarcinoma; some strains also have medullary thyroid carcinoma and/or islet cell hyperplasia ^a	Death at E11–E15; abnormal proliferation and cell death in haematopoietic and nervous systems ^a
<i>WT1</i>	Wilms' tumour ^a	Normal ^a	Death at E13.5; heart abnormal, no gonads or kidneys ^a
<i>p53</i>	Li–Fraumeni syndrome: spectrum of tumours, most commonly of breast or brain ^a	Spectrum of tumours, predominantly soft tissue sarcomas ^a	Some lethality with exencephaly in female embryos. Early postnatal development of tumours, primarily lymphomas ^a
<i>NF1</i>	Neurofibromatosis (multiple cutaneous and plexiform neurofibromas, café au lait spots, iris hamartomas) ^a	Tumours including pheochromocytoma, myeloid leukaemia, lymphoma, lung adenocarcinoma ^a	Death at midgestation, heart abnormal ^a
<i>BRCA1</i>	Breast cancer ^a	Normal ^{b–e}	Death at E7.5–E13; retarded, abnormal proliferation and cell death in neuroepithelium ^{b–e}
<i>BRCA2</i>	Breast cancer ^f	Normal ^g	Death at E8.5–E9.5; retarded and disorganized ^{e,g}
<i>APC</i>	Familial adenomatous polyposis (adenomas, principally of colon, some progressing to carcinoma) ^a	Polyps of intestinal tract, principally small intestine, some progressing to carcinoma ^a	Death before E11; abnormal development of primitive ectoderm ^a
<i>MSH2</i>	HNPCC (hereditary non-polyposis colorectal cancer) ^{h,i}	Elevated tumour incidence at various sites, not including intestine ^j	Lymphoma ^{j–l}
<i>MSH6</i>	HNPCC ^m	Normal ⁿ	Adenomas and carcinomas of duodenum, jejunum and ileum, and B and T cell lymphomas ⁿ
<i>MLH1</i>	HNPCC ^{o,p}	No phenotype reported ^q	Sterile; meiotic abnormalities ^q
<i>PMS2</i>	HNPCC ^r	Sarcomas, lymphomas ^s Sterile, abnormal meiotic chromosome synapsis ^s	
<i>DPC4</i>	Juvenile polyposis ^t	Normal ^u	Death before E7.5; abnormal visceral endoderm ^u
<i>VHL</i>	von Hippel–Lindau syndrome (retinal angioma, hemangioblastoma of CNS, pheochromocytoma, renal cell carcinoma, pancreatic cancer) ^a	Normal ^v	Death between E10.5 and E12.5; defective vasculogenesis in placental labyrinth ^v
<i>E-CAD</i>	Familial gastric cancer ^w	Normal ^{x,y}	Death at E4–5; failure to form trophectoderm and blastocoel ^{x,y}

Gene designations are for the human gene in each case, the mouse genes being written with an initial capital only, e.g. *Rb-1*. Phenotypes listed are for null, or presumed null, mutations; some phenotypes exhibit incomplete penetrance. References: ^aWilliams and Jacks (1996) and references cited therein; ^bGowen *et al.*, 1996; ^cHakem *et al.*, 1996; ^dLiu *et al.*, 1996; ^eLudwig *et al.*, 1997; ^fWooster *et al.*, 1995; ^gSuzuki *et al.*, 1997; ^hLeach *et al.*, 1993; ⁱFishel *et al.*, 1993; ^jde Wind *et al.*, 1998; ^kde Wind *et al.*, 1995; ^lReitmair *et al.*, 1995; ^mMiyaki *et al.*, 1997; ⁿEdelmann *et al.*, 1997; ^oBronner *et al.*, 1994; ^pPapadopoulos *et al.*, 1994; ^qBaker *et al.*, 1996; ^rNicolaides *et al.*, 1994; ^sBaker *et al.*, 1995; ^tHowe *et al.*, 1998; ^uSirard *et al.*, 1998; ^vGnarra *et al.*, 1997; ^wGuilford *et al.*, 1998; ^xLarue *et al.*, 1994; ^yReithmacher *et al.*, 1995.

exception of the mismatch repair genes *Msh2*, *Msh6*, *Mlh1* and *Pms2*, which fall into the caretaker category, and *p53*, which possesses both caretaker and gatekeeper functions, mice homozygous for null mutations in the genes listed in Table I all die *in utero*, showing that these genes have essential functions other than protection against cancer. Examination of the abnormal embryos gives important clues as to what these functions are. Heterozygotes, on the other hand, exist in both mice and humans. Not surprisingly, in view of the large number of differences between these species, there are both parallels and contrasts in phenotype. One difference relevant to all these genes is that mice are smaller and shorter-lived than humans, so that the probability of a somatic mutation occurring in at least one cell of a susceptible tissue is correspondingly

less. Other differences relate specifically to individual genes. Where there are differences between human and mouse phenotypes, identification of the interspecies difference that is responsible can give valuable information about the mechanistic basis of the disease. Where there are parallels, the mouse can provide a useful model for experimental intervention. In this review I shall illustrate these points using *RB-1* and *WT1* as examples.

The *RB-1* gene

The tumour suppressor gene paradigm is the retinoblastoma susceptibility gene *RB-1*. Retinoblastomas are childhood retinal tumours of which about 40% of all cases are familial. More than 90% of individuals constitutively

Fig. 1. Strategies for producing animals of various genetic constitutions by gene targeting. Heterozygous (+/-) ES cells produced by a single round of gene targeting can be injected into wild-type blastocysts to produce chimeras containing heterozygous and wild-type cells (+/- ↔ +/+) which on breeding produce in the first-generation heterozygous (+/-) mice and in the second-generation homozygous (-/-) mice if these are viable. ES cells with both alleles mutated (-/-) can be generated either by two sequential targeting steps or by high-geneticin selection from heterozygous cells. As discussed in the text, the two routes may produce cells with different properties. Injection of these cells into blastocysts produces -/- ↔ +/+ chimeras which in many cases survive longer than -/- mutants, allowing the properties of -/- cells to be studied at later stages of development. For simplicity, targeted alleles are shown as null alleles, but the strategies for the introduction of alleles with more subtle modifications such as floxing (see text) are identical.

heterozygous for an *RB-1* mutation develop retinoblastoma as a result of a somatic event occurring in one or more cells that eliminates the function of the wild-type allele, usually by allele loss (reviewed by Knudson, 1993). There are several candidates for the cell type of origin of retinoblastoma, including primitive neurotubular cells, glial cells and photoreceptor cells (reviewed by Hooper, 1994). In addition to retinal tumours, about 15% of *RB-1* heterozygotes develop osteosarcomas. *RB-1* allele loss also occurs in sporadic lung, breast, prostate and bladder carcinomas, although no increase in incidence of these

Fig. 2. Conditional gene targeting. Floxed (f) and null (-) alleles produced by gene targeting in ES cells are introduced separately into the germline as in Figure 1, and mice containing one floxed allele and one null allele (-f) produced by breeding. These mice are then mated to transgenic mice that express cre recombinase either tissue-specifically (as indicated by the white area), or inducibly under the control of the experimenter (or in some cases both tissue-specifically and inducibly). Where cre recombinase is functional it converts the normally functioning floxed allele into a mutant allele (shown here as a null allele, but other modifications can be designed). Alternatively, mice homozygous for the floxed allele may be mated to cre-expressing transgenic mice, but recombination in both alleles is then required to produce homozygous mutant cells.

tumours is seen in germline heterozygotes (see Knudson, 1993).

The *RB-1* gene codes for a 105 kDa protein present in most cell types which undergoes cell-cycle-dependent changes in phosphorylation (Mulligan and Jacks, 1998). The hypophosphorylated protein associates with the cell nucleus and binds through a bipartite domain termed the 'pocket' to transcription factors such as members of the E2F family, 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, which include several whose products are required during S phase, is inhibited by the binding of the *RB-1* gene product; the mechanism involves recruitment of histone deacetylase bound through the pocket domain (Brehm *et al.*, 1998; Magnaghi-Jaulin *et al.*, 1998). E2F family members differ in their binding preference for the *RB-1* gene product or one of two other related proteins, p107 or p130; different interactions predominate at different cell-cycle phases, and it is likely that this provides a further level at which the expression of E2F-dependent genes can be regulated. The phosphorylation of the *RB-1* gene product is mediated by G₁ cyclin-dependent kinases such as cyclin E-CDK2 and cyclin D-CDK4, the latter being subject to inhibition by the cdk inhibitor p16^{INK4A}. The significance of this growth-control pathway is emphasized by the frequent occurrence of mutations not only in *RB-1* but also in the genes encoding p16^{INK4A}, cyclin D and CDK4 in human cancer (Mulligan and Jacks, 1998).

Mice heterozygous for a null mutation in the corresponding gene, *Rb-1*, do not develop retinoblastomas but do develop pituitary adenocarcinomas (Jacks *et al.*, 1992; Hu *et al.*, 1994; Williams *et al.*, 1994a; Harrison *et al.*, 1995). These tumours develop from the melanotroph cells in the intermediate lobe, a structure which is present only in vestigial form in adult humans, and this probably underlies the absence of corresponding tumours in humans. In cross-bred mice, i.e. mice that result from the intercrossing of two inbred strains, the development of these tumours

results in shorter lifespans in heterozygotes that inherit the mutant allele paternally than in those that inherit it maternally (Harrison *et al.*, 1995; Nikitin *et al.*, 1997). The latter authors found, however, that parental origin did not influence *Rb-1* expression itself, and that *Rb-1* homozygotes rescued by a human *RB-1* transgene had similar survival rates irrespective of the parental origin of the transgene. They proposed the existence of imprinting that affected not the *Rb-1* gene itself but a linked locus. This leads to the prediction that there should be no effect of parental origin of the mutant allele in inbred mice, and this is borne out by experiment (Armstrong and Hooper, 1998). In humans, imprinting appears to play a role in the development of sporadic osteosarcomas but not of retinoblastomas (Toguchida *et al.*, 1989). In addition to the pituitary tumours some, but not all, sublines of *Rb-1* heterozygotes develop medullary thyroid carcinoma (Williams *et al.*, 1994a; Harrison *et al.*, 1995). The difference between the sublines may be a consequence of differences in the targeted allele, in the genetic background or in environmental exposure.

Homozygous *Rb-1* mutant embryos fail to survive to term, and die at various ages shortly after midgestation. The variability in the length of survival is seen in both inbred and cross-bred mice and is therefore not attributable to differences in genetic background (A.R.Clarke and J.F.Armstrong, manuscript in preparation). At midgestation they show abnormalities in the haematopoietic and nervous systems, in both cases involving increased levels of apoptotic cell death and overabundant or ectopic mitosis (reviewed by Hooper, 1996). The tissues affected normally show high-level *Rb-1* expression and are those in which a mitotically active precursor cell population matures earliest in embryonic development to a post-mitotic differentiated cell. This suggests a generic role for *Rb-1* 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 characterized by reduced levels of both mitosis and apoptosis (Bellamy *et al.*, 1995).

Effects of homozygosity on events occurring later in development have been studied in chimeras produced by injecting sequentially targeted *Rb-1*^{-/-} cells into wild-type blastocysts (Robanus-Maandag *et al.*, 1994; Williams *et al.*, 1994b). The *Rb-1*-null cells contributed, albeit in some instances at reduced levels, to all adult tissues examined. No retinoblastomas were seen in these chimeras, 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.

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 (Hooper, 1994), of which one is that laboratory mice differ from humans in the level of exposure of their retinas to sunlight. It is therefore intriguing that the incidence of retinoblastoma in human populations at different geographical locations increases significantly with the ambient erythral dose of ultraviolet B radiation from sunlight, and that this effect is seen only for unilateral and not for bilateral cases, as would be predicted from the hypothesis is that it is due to an effect of sunlight on somatic events

leading to *RB-1* inactivation (Hooper, 1998). However, differences in exposure to ultraviolet radiation between mice and humans are alone insufficient to account for the lack of retinoblastomas in *Rb-1*^{+/-} mice, since controlled exposure of the mice to fluorescent light with a daylight spectrum has not led to the development of any retinoblastomas in the exposed mice (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 possible need in the mouse for an additional genetic event or events, and indeed it has recently been reported (Robanus Maandag *et al.*, 1998) that retinoblastomas developed in six of 14 chimeric 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 required mutation of *p107* and probably a further event involving an unidentified gene that occurred somatically in the chimeras. 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, these studies illustrate how differences between human and mouse phenotypes can generate testable hypotheses that can lead to an increase in knowledge about human cancer.

The *WT1* gene and Denys–Drash syndrome

The Wilms' tumour suppressor gene, *WT1*, encodes a nuclear protein with structural motifs characteristic of transcription factors, including four C-terminal zinc fingers (Hastie, 1994). There exist 16 isoforms of this protein resulting from a combination of alternative initiation codon usage, RNA editing and alternative splicing at two sites, one determining whether the whole of exon 5 is included and the other affording the option of inserting three amino acids (KTS) between zinc fingers 3 and 4. These isoforms bind not only to DNA but also to RNA, and a further role in RNA splicing is suggested by co-localization of the +KTS isoforms with splicing factors in a speckled nuclear pattern (reviewed by Pritchard-Jones, 1997). *WT1* dysfunction is implicated in the aetiology of certain Wilms' tumours in the kidney. Wilms' tumours mimic in histological appearance cell types and structures seen in normal fetal kidney development, and about 2% occur in association with a defined malformation syndrome. WAGR syndrome (Wilms' tumour, aniridia, genitourinary malformation and mental retardation) occurs in children heterozygous for an interstitial deletion which encompasses both *WT1* and the aniridia gene *PAX6*. Children with Denys–Drash Syndrome (DDS), a rare childhood disease characterized by nephropathy involving mesangial sclerosis, associated with genital anomalies (notably XY pseudohermaphroditism and gonadal dysgenesis) and Wilms' tumour (Denys *et al.*, 1967; Drash *et al.*, 1970), are constitutionally heterozygous for *WT1* point mutations affecting the zinc finger domain (Little and Wells, 1997). A phenotype similar to DDS but without Wilms' tumour is shown by patients with Frasier syndrome, who are heterozygous for splice site mutations that eliminate the +KTS isoform (van Heyningen, 1997).

A deletion comparable to that of WAGR syndrome is

present in the *Sey^{De}* (Dickie's small-eye) mouse, but mice heterozygous for this deletion do not develop Wilms' tumour (Glaser *et al.*, 1990). This is also the case with mice heterozygous for a null targeted allele of *Wt1* (Kreidberg *et al.*, 1993). Embryos homozygous for this allele die in the second half of gestation and exhibit abnormalities of heart development and total failure of kidney and gonad development.

There are a number of features of Denys–Drash syndrome that are still unexplained. How the *WT1* mutations present in DDS exert a dominant effect is not well understood, and we do not know in what cell type in the kidney the mutations exert their primary effect, why they exhibit incomplete penetrance and variable expressivity, or why DDS is a progressive disease. These questions cannot be addressed in human patients, and so my laboratory, in collaboration with that of Prof. Nicholas Hastie, has used gene targeting to generate mouse embryonal stem cells carrying a DDS-type mutation in one allele of their *Wt1* gene (Patek *et al.*, 1998; C.E.Patek, M.H. Little, S.Fleming, C.Miles, J.-P.Charlieu, A.R.Clarke, K.Miyagawa, S.Christie, J.Doig, D.J.Harrison, D.J. Porteous, A.J.Brookes, M.L.Hooper and N.D.Hastie, submitted for publication). When chimeras produced by injecting these cells into wild-type blastocysts were mated to wild-type mice, only one transmitted the mutant allele to one of its progeny, a sterile XXY male. The chimera that transmitted the mutation was a female and must therefore have had the sex chromosome constitution XX↔XY, since the ES cells used for targeting were XY. Failure to transmit via male chimeras could be attributed to gonadal abnormalities (see below). The XXY constitution of the progeny heterozygous male was probably due to failure of X–Y segregation at meiosis in the oocyte (cf. Bronson *et al.*, 1995). This heterozygous mouse developed outward signs of disease and on autopsy exhibited nephropathy typical of DDS. That this was due to a dominant effect of the mutation was confirmed by analysing chimeras containing heterozygous cells: none developed overt signs of illness, but the majority of those autopsied at >6 months of age showed focal and segmental mesangial sclerosis. However, this was not seen in chimeras autopsied in the first month of postnatal life, showing that the disease develops progressively as in human DDS.

In one chimera, one kidney was replaced by a Wilms' tumour derived predominantly from the heterozygous cells, which had acquired a further lesion that caused skipping of the exon coding for zinc finger 3 in the transcript from the non-targeted allele, which is predicted to result in a non-functional protein (Haber *et al.*, 1992). Thus, as is usually the case in human DDS (Little and Wells, 1997), both *Wt1* alleles are functionally inactivated in the tumour.

Interpretation of the gonadal phenotype of these mice is complicated by their sex chromosome constitution, but a number of features were present in male chimeras that cannot be explained by sex chromosome constitution and are consistent with effects seen in human DDS patients: these included instances of failure of Sertoli cell maturation, micropenis and complete absence of gonads. The three characteristic features of DDS, namely mesangial sclerosis, Wilms' tumour and gonadal abnormalities, are thus all represented in these animals.

The mesangial sclerosis seen in these mice does not develop in mice heterozygous for a null allele of *Wt1* (Glaser *et al.*, 1990; Kreidberg *et al.*, 1993), and therefore cannot be attributed to haploinsufficiency. The DDS mutant allele must therefore act either by a dominant-negative (antimorphic) or a gain-of-function (neomorphic) mechanism. As the WT1 protein is known to self-associate through its N-terminal region the model currently favoured is that DDS mutations act by a dominant-negative mechanism. The simplest hypothesis would be that the active form of WT1 is the dimer, that the protein exists predominantly in this form in the cell and that the mutant protein associates with the wild-type to form inactive heterodimers. However, both in heterozygous ES cells and in the Wilms' tumour, the DDS mutant WT1 protein constituted <5% of the total WT1 protein, although its transcript was present at the same level as that from the other allele. As this appears not to be a tissue-specific phenomenon we assume that it is also true in the kidney, but we have not yet been able to confirm this because chimerism and low levels of expression make it technically more difficult. If it is indeed the case, this implies that only a small amount of mutant protein is sufficient to disrupt urogenital function and would argue against the above-mentioned simple hypothesis. The model could, however, be retained in one of two modified forms. The first possibility is that for wild-type WT1 the equilibrium is in favour of the inactive, monomeric form and only small amounts of active dimer are present. It is known that the heterodimeric association between mutant and wild-type proteins is stronger than the homodimeric association of wild-type protein (Moffett *et al.*, 1995), so that a small amount of mutant protein may produce heterodimers at a concentration capable of competing effectively with the homodimer for its target. The second possibility is that the active form is a higher oligomer than the dimer so that a single mutant subunit can sequester many wild-type subunits into inactive oligomers. Nevertheless, alongside these alternative possibilities for dominant-negative action, the possibility that DDS mutations act by a gain-of-function mechanism should not be neglected.

Having demonstrated a causal link in mice between our mutant *Wt1* allele and the three features typical of Denys–Drash syndrome, we are currently exploring various approaches, including the use of conditional gene targeting (Figure 2), that should enable the difficulties in germline transmission of this mutant allele to be circumvented. This should provide a model to enable us to investigate how the mutant allele acts, why DDS is a progressive disease and why it exhibits low penetrance and variable expressivity. It should also facilitate identification of the *in vivo* target genes of WT1, thereby contributing to a molecular understanding of WT1 function and its role in glomerular development and Wilms' tumorigenesis.

Concluding remarks

The work reviewed here illustrates how both differences and similarities between mice and humans with similar tumour suppressor genotypes can be of value in illuminating human disease mechanisms. With the application of gene targeting technology to more tumour suppressor genes and the ever-increasing sophistication in the types

of modification that can be introduced, we can anticipate an explosion in our understanding of the mechanistic basis of different types of cancer, with all that this implies for the implementation of improved preventive and therapeutic strategies.

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