Tumour suppressor genes

J. K. Cowell
ICRF Oncology Group, Institute of Child Health, London, U.K.

Summary. Genes responsible for the hereditary predisposition to a variety of human cancers have now been isolated. Their function seems to be part of complex signalling pathways involved in the control of cellular differentiation and the cell cycle. The presence of a single copy of these genes appears to be sufficient to ensure normal development, i.e. prevents tumorigenesis, and has earned them the name - tumour suppressor genes.

The concept

The malignant phenotype is inherited faithfully from cell to cell demonstrating that it is genetically determined. The idea that this phenotype could be suppressed came from somatic cell hybrid studies where malignant cells were fused with normal cells. The general finding was that normal cells could suppress the malignant phenotype [1, 2]. Variants, however, could emerge from the hybrid cell populations which were again tumorigenic. Detailed cytogenetic analysis of these hybrids showed that they had lost specific chromosomes, which presumably carried the genes which could suppress tumour formation - tumour suppressor genes [3]. Using microcell chromosome transfer Stanbridge and colleagues were able to introduce individual chromosomes into tumour cells and, in a series of experiments, demonstrated that the malignant phenotype of specific tumour cell types could be suppressed by different chromosomes [3]. The inference behind these observations was that tumour cells have lost the function of specific genes which the normal chromosomes could provide, re-establishing normal regulatory controls within the cell. As such, the malignant phenotype is recessive at the cellular level and the genes responsible can be referred to as 'recessive oncogenes'. It also became clear, during the course of cell hybridisation experiments, that differentiation could override the malignant phenotype [4, 5]. Thus, when highly differentiated cells were fused with malignant cells the resultant hybrids assumed the differentiated phenotype. In this situation, rather than compensating for the specific defect promoting the malignant phenotype the genes controlling malignancy are shut down non-specifically. Other indications of the recessive nature of the malignant phenotype came from a study of human hereditary cancer syndromes particularly the rare childhood eye cancer, retinoblastoma.

Retinoblastoma

Retinoblastoma (Rb) results from the failure of immature retinal cells to differentiate into photoreceptor cells. About 12% of patients have a strong family history of Rb where the tumour phenotype segregates as an autosomal dominant trait [6]. However, not all retinal precursor cells develop into tumour cells and so it is only a predisposition to tumorigenesis that is inherited. In an epidemiological study of Rb, Knudson [7] showed that, in individuals with a germline mutation, only a single additional event was required for tumour initiation and this event occurred randomly. In sporadic cases both events occurred sporadically in the same immature retinal cell. The assumption was that mutation of both copies of a single gene was the rate limiting step for Rb tumorigenesis [8]. It was not until twelve years later that this theory was formally proven. The Rb gene was known to be in chromosome region 13q14 from an analysis of patients with constitutional chromosome deletions and linkage studies using chromosome 13 markers [9, 10]. Several groups identified patients who were constitutionally heterozygous for markers around the 13q14 region and showed that their tumours had lost one of these alleles [11-13]. Furthermore, when tumours from patients with a germline Rb mutation were analysed [14], it was the allele inherited from the affected parent which was retained. This loss of heterozygosity (LOH) could occur as a result of deletion, chromosome non-disjunction or mitotic recombination [12]. By these mechanisms the duplication of the initial mutation, which occurs in 70% of tumours [12, 15], results in loss of function of the Rb gene in the cell. In 1986, following a series of now well documented experiments [16], the retinoblastoma susceptibility gene (RB1) was cloned [17]. DNA sequence analysis of RB1 in tumour cells confirmed the homozygous nature of the mutations [18, 19] and identified the genetic lesions...
in normal cells from genetically predisposed individuals [20, 21]. The majority of mutations are deletions, insertions and point mutations which result in the generation of premature stop codons, but occasionally missense mutations were identified [22] which were apparently associated with 'mild' forms of the disease.

The RB1 gene encodes a nuclear phosphoprotein, pRB, which participates in the control of the cell cycle. The underphosphorylated form of pRB apparently maintains cells in quiescence [23] and phosphorylation accompanies the transition from G1 → S phase. An interesting early observation was that the transforming oncoproteins of dominantly transforming DNA tumour viruses, such as the E1a protein of adenovirus [24], large T (LT) antigen of SV40 [25] and E7 protein of papilloma virus [26] bind to pRB. It is the underphosphorylated form which is targeted by the viral oncoproteins thus, by sequestering pRB, the repression on cell division is lifted. The situation is not so simple, however, since it is clear that pRB must complex with other cellular proteins involved in the cell cycle to exert its effect [16]. Particularly important are proteins directly involved in cell division such as the E2F transcription factor, cyclin A and Cdc2 kinase. pRB also complexes with a developmentally regulated transcription factor (DRTF) which may give clues to its role in cell differentiation.

The analysis of Rb established many of the prece-dents for the study of human hereditary cancers, the assumptions being that they too resulted from the loss of genes important in the control of cellular differentiation. LOH was one particularly important observation.

**Loss of heterozygosity**

Linkage studies provide the only true indication for the location of genes predisposing to cancer. However, this approach requires that there are sufficient numbers of families with a clear pattern of inheritance, which is not always the case. Loss of heterozygosity, however, has also been used to locate the site of potential recessive cancer genes. Where the observation of LOH is supported by family linkage data, this assumption is more valid. The generally accepted implication behind the observation of LOH is that recessive mutations, which contribute to the development of the malignant phenotype, are being exposed. Since the early studies in Rb [11, 12] there has been a vast amount of literature on the subject describing LOH events in a wide variety of different tumour types. The net result of this large survey is that specific chromosome loci are apparently involved in the development of different tumours [3, 27]. Rb, however, was exceptional with LOH restricted to a single chromosome, the majority of tumours showing LOH for a variety of different chromosomes, possibly supporting a multistep process en route to tumorigenesis. Random loss of chromosomes, however, is a hallmark of tumour cells and the exposure of any recessive mutation which allows the cells to grow better will have a selective advantage. Many of these changes, however, will not be related to the initial, all important, events leading to tumorigenesis.

**Wilms' tumour**

In the paediatric kidney cancer, Wilms' tumour (WT), there was evidence from cytogenetic analysis of constitutional chromosome abnormalities in patients with other congenital abnormalities that region 11p13 was the site of the predisposition gene [28]. Furthermore, Knudson and Strong [29] had suggested that WT, like Rb, also results from two mutations and LOH was demonstrated for the short arm of chromosome 11 [30, 31]. It became clear later, however, that whilst many tumours showed LOH for the 11p13 region, others showed LOH in 11p15, suggesting the location of a second WT gene [32, 33]. To complicate matters further, linkage analysis in a few rare instances, where the tumour phenotype apparently segregated as an autosomal dominant, failed to show any linkage with 11p markers suggesting that there was a third WT gene [34, 35]. A candidate gene, WT1 [36], was cloned from the 11p13 region [37, 38] which occasionally showed structural rearrangement in tumour cells [39-41]. The gene was expressed largely in the developing kidney and gonads, particularly when cells made the transition from mesenchyme to epithelium [42].

The function of the WT1 gene is largely unknown but it has a zinc finger (ZF) motif which implies that it might regulate the expression of genes important in the normal differentiation of the kidney. The majority of mutations within the WT1 gene, so far reported, are located within this ZF region and presumably result in loss of its ability to regulate the developmental genes under its control. The ZF domains of WT1 show considerable homology with early growth response (EGR) genes which appear during G0 and G1 phases of the cell cycle. Both WT1 and EGR recognise similar DNA binding sites [43] and are possibly involved in nuclear signal transduction. The kidneys and gonads are derived from embryologically adjacent tissues and patients with 11p13 deletions can develop gonadoblastoma instead of WT [44]. Patients with 11p13 deletions also show abnormal development of the genital system and, since the WT1 gene is expressed at high levels in the developing gonads, this suggests a role for WT1 in the developing genital system. Patients with Denys-Drash syndrome have abnormal genitalia and are also predisposed to WT [45]; these patients have been shown to have constitutional mutations in the WT1 gene but not all of them develop WT [46]. Thus, although it clearly plays a role in the differentiation of both tissues, the genital system seems to be more sensitive to WT1 mutations than the kidneys, which possibly reflects the fact that more than one gene is involved in Wilms' tumorigenesis.
Neurofibromatosis

Neurofibromatosis type 1 (NF1) – formerly Von Recklinghausen's neurofibromatosis – is inherited as an autosomal dominant disorder with an incidence of 1 in 3000. The clinical phenotype is variable, ranging from hyperpigmented patches of the skin to multiple tumours of the peripheral nerves and brain suggesting it is important in the differentiation pathway for nerve cells. Linkage analysis assigned the NF1 gene to chromosome region 17q12-q22 [47, 48]. Following now standard procedures of positional cloning around translocation breakpoints [49, 50] the NF1 gene was cloned. The presence of point mutations in this gene in predisposed individuals provided strong support for its authenticity [50].

The NF1 gene product [51] is structurally related to the GTPase activating protein (GAP) which interacts with the ras protooncogene product, p21. Although its mode of action is not fully understood [52], p21 operates within the cytoplasm at the plasma membrane interface and appears to be activated by incoming signals which it then relays to other molecules downstream in the pathway to cell proliferation. The GAP proteins can bind to p21 either to maintain it in a quiescent state or activate it into a signal-transmitting state. Oncogenic mutations of ras escape the down-regulation by GAP maintaining it in an active state and causing constant transmission of growth stimulating signals. Although it is still not clear how NF1 acts in this pathway one suggestion is that it binds to p21 [53] preventing normal single transduction. When NF1 is missing, ras function is not suppressed, and one route to the malignant phenotype is opened.

Familial adenomatous polyposis

Familial adenomatosis polyposis (FAP) – originally reported as polyposis coli – is a dominantly inherited disorder which predisposes to the development of colon cancer and the tumour phenotype is linked to markers on chromosome 5 [54]. LOH was also observed for 5q21 markers in 30% of tumours [55]. It is clear that this gene must be inactivated for the development of colon carcinomas but, as shown by Vogelstein and colleagues, several other events must also occur [56]. The development of colon carcinoma can be followed through a series of 4 stages, from hyperplastic changes in the epithelium, to adenomatous polyps, to non-invasive carcinoma and finally invasive carcinomas. Analysis of each of these stages is possible. The majority of large adenomas have an activated K-ras oncogene, as well as LOH for chromosome 5. The progression to carcinomas is accompanied by loss of sequences on chromosomes 17 and 18. It was shown that the loss of alleles on chromosome 17 was a reflection of mutations in the p53 gene (see below). The locus implicated by LOH studies on chromosome 18 lay in region 18q21-qter and two carcinomas showed homozygous deletions of sequences in region 18q21.3. A cDNA was subsequently isolated from this region which was shown to have characteristics of a cell surface adhesion molecule [57] and was termed DCC (deleted in colon cancer). The consensus finding is that the molecule is part of a pathway transferring extra cellular signals into the cell, loss of which allows uncontrolled proliferation. Following identification of the FAP locus, on chromosome 5, DNA from the appropriate region was isolated and several genes identified [58]. One gene, MCC (mutated in colon carcinoma), showed mutations in tumour cells possibly implicating it in development of these tumours [59] but Joslyn et al. [60] later showed it lay outside the critical region. A second gene, APC (adenomatous polyposis coli), was shown to be mutated in the gene line of patients predisposed to FAP [61] strongly supporting its role in tumorigenesis. The function of these genes is not clear yet but APC has some structural morphology with cellular G-proteins which are connected with cellular signal transduction through their association with ras and NF1.

p53

The p53 protein was originally identified as part of a complex with the oncogenic protein of SV40 virus [62]. Despite early misleading observations, wild-type p53 was shown to have growth suppressing ability. The p53 gene is located in chromosome region 17p which shows frequent LOH in a wide variety of tumours. In some tumours loss of p53 allows expression of the malignant phenotype whereas, in other tumours, mutations stabilise the p53 protein which then promotes cell growth. Although p53 has many similarities with RB1 [63], mutant p53 can promote cell growth even in the presence of the wild type gene. The frequent involvement of LOH and mutations of p53 in human cancer suggests a non-specific role. Indeed, in the development of colon cancer, for example, it is probably only important in tumour progression. Constitutional p53 mutations were found in patients with Li-Fraumeni syndrome, an inherited cancer susceptibility condition. Affected individuals develop multiple primary tumours in the second and third decades of life [64] in a variety of different tissues, presumably after the wild type gene is lost.

The p53 protein is phosphorylated in a cycle-dependent manner [65]. It accumulates in the cytoplasm at the start of G1 and moves to the nucleus at the beginning of S-phase [66]. The possible functions, therefore, are transactivation of transcription or regulation of DNA replication [52]. Introducing the wild type p53 into transformed cells arrests them in G1. Although p53 is probably centrally involved in the control of the cell cycle it is not required for normal development as shown by the generation of nullizygous transgenic mice.
band 3p21, is consistently found in tumours and cell lines and LOH is also observed for this chromosome. Deletion on the short arm of chromosome 3, involving interpretation of family pedigree studies. Despite this, a example, is associated with smoking which modifies the human cancers is more complicated. Lung cancer, for chromosome 22. The genetic analysis of the more common neurofibroma gene, NF2 [72], is thought to be on chromosome 1 [69]. Linkage some of the requirements of the two-hit hypothesis. Disruption of the function of certain proteins, which have a variety of functions although they loosely appear to be participants in signalling pathways related to cellular growth control. Signals received by the cell must be passed across the membrane, through the cytoplasm and into the nucleus where cell division is initiated. Disruption of the function of certain proteins, which are essential for the normal control of cellular proliferation, sends the cell inextricably down the road to tumorigenesis.

Although the search for recessive oncogenes has been successful there are still those which have not as yet been isolated but whose location is known. The neuroblastoma gene for example, which also fulfils some of the requirements of the two-hit hypothesis [68], is thought to be on chromosome 1 [69]. Linkage analysis has also identified genes on chromosome 11 [70] and 10 [71] associated with multiple endocrine neoplasia types 1 and 2 respectively and the acoustic neurofibroma gene, NF2 [72], is thought to be on chromosome 22. The genetic analysis of the more common human cancers is more complicated. Lung cancer, for example, is associated with smoking which modifies the interpretation of family pedigree studies. Despite this, a deletion on the short arm of chromosome 3, involving band 3p21, is consistently found in tumours and cell lines and LOH is also observed for this chromosome. Recently a gene from this region has been identified [73] which is a protein tyrosine phosphatase, a class of genes which have also been implicated in the complex control of the cell cycle. Although no candidate gene is available for hereditary breast cancer, as yet, the complexities of genetic analysis have been largely overcome and a locus in chromosome region 17q21 identified [74]. Undoubtedly the application of reverse genetic procedures will soon result in the molecular cloning and characterisation of these genes which will complete more of the picture about the role of tumour suppressor genes in the development of human cancer.

The clinical benefit of the isolation of tumour suppressor genes is that prenatal screening is possible for those with a family history, using standard linkage analysis [75]. Where the phenotype is variable, or the penetrance incomplete, mutant gene carriers can be identified unequivocally removing the need for unnecessary screening. The availability of the causative gene, and techniques to identify heterozygous mutations in constitutional cells [21], will allow prenatal screening for the first born children of patients carrying de novo germline mutations. The ability to predict in the future exactly who is at risk to tumour formation will undoubtedly greatly improve the clinical management of individuals with hereditary cancer predisposition syndromes.

References

14. Caveen WK, Hansen MF, Nordenskjold M et al. Genetic ori-
   gin of mutations predisposing to retinoblastoma. Science 1985;
   228: 501-3.
15. Zhu X, Dunn JM, Phillips RA et al. Preferential germline muta-
   tion of the paternal allele in retinoblastoma. Nature 1989; 340:
   312-3.
16. Cowell JK, Hogg A. The genetics and cytogenetics of reti-
   with properties of the gene that predisposes to retinoblastoma
18. Dunn JM, Phillips RA, Becker A et al. Identification of germ-
   line and somatic mutations affecting the retinoblastoma gene.
19. Dunn JM, Phillips RA, Zhu X et al. Mutations in the RB1 gene
   and their effects on transcription. Mol Cell Biol 1989; 9:
   4596-604.
20. Vandell DW, Campbell TA, Dayton SH et al. Oncogene point
   mutations in the human retinoblastoma gene: their application
   mutations in the RB1 gene in retinoblastoma patients using
   single strand conformation polymorphism (SSCP) analysis and
22. Onadim Z, Hogg A, Baird PN et al. Oncogene point mutations
   in exon 20 of the RB1 gene in families showing incomplete
   penetrance and mild expression of the retinoblastoma pheno-
23. Mihara K, Cao X-R, Yen A et al. Cell cycle-dependent regula-
   tion of phosphorylation of the human retinoblastoma gene
   between an oncogene and an anti-oncogene: the adenovirus
   E1A proteins bind to the retinoblastoma gene product. Nature
25. DeCaprio JA, Ludlow JW, Figge J et al. SV40 large tumour
   antigen forms a specific complex with the product of the reti-
26. Dyson N, Howley PM, Munger K et al. The human papilloma
   virus-16 E7 oncoprotein is able to bind to the retinoblastoma
27. Weinberg RA. Tumour suppressor genes. Science 1991; 254:
   1138-45.
28. Riccardi VM, Sujansky E, Smith AC et al. Chromosome im-
   plication in the aniridia-Wilms' tumour association: 11p intersti-
29. Knudson AG, Strong LC. Mutation and cancer: a model for
   Wilms' tumour of the kidney. J Natl Cancer Inst 1972; 40:
   313-53.
30. Rowe LD, Hogg A, Baird PN et al. Germline and somatic mutations
   involving the Wilms' tumour suppressor gene. Hum Genet
   Wilms' tumour gene is involved in genitourinary development.
34. Shalkoff BR, Rouleau GA, Ozolius LJ et al. Genetic linkage of
   von Recklinghausen neurofibromatosis to the nerve growth factor
35. Pritchard-Jones K, Fleming S, Davidson D et al. The candidate
   Wilms' tumour locus is adjacent to the EGR-1 consensus
36. Zhu X, Dunn JM, Phillips RA et al. Evidence for WT1 as a
   Wilms' tumour (WT) gene: intragenic germinal deletion in bi-
37. Pritchard-Jones K, Fleming S, Davidson D et al. The candidate
   Wilms' tumour gene is involved in genitourinary development.
41. Solomon E, Voss R, Hall V et al. Chromosome 5 allele loss in
42. Vogelstein B, Fearon ER, Hamilton SR et al. Genetic altera-
   tions during colorectal-tumour development. N Eng J Med
43. Fearon ER, Cho KR, Ngo JM et al. Identification of a chro-
   mosome 18q gene that is altered in colorectal cancers. Science
44. Kinzler KW, Nilbert MC, Su L-K et al. Identification of FaP
   locus genes from chromosome 5q21. Science 1991; 253:
   661-5.
45. Kinzler KW, Nilbert MC, Vogelstein B et al. Identification of a
   gene located at chromosome 5q21 that is mutated in colorectal
46. Joslyn G, Carlson M, Thliveris A et al. Identification of dele-
   tions and three new genes at the familial polyposis coli
47. Nishisho I, Nakamura Y, Miyoshi Y et al. Mutations of chro-
69. Michalski AJ, Cotter FE, Cowell JK. Isolation of chromosome-specific DNA from an Alu polymerase chain reaction library to define the breakpoint in a patient with a constitutional translocation t(1;13)(q22;q12). Oncogene 1992, in press.

Received 11 May 1992; accepted 22 May 1992.

Correspondence to:
John K. Cowell, Ph.D.
ICRF Oncology Group
Institute of Child Health
30, Guilford Street
London, WC1, U.K.