CANCER:
THE MISSING GENE HYPOTHESIS

Medical Grand Rounds

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We are beginning to learn precisely how genes convert normal cells into cancer cells. First to be discovered were the viral oncogenes, pirated copies of cellular genes that are subverted by tumor-causing viruses. A single copy can transform a normal cell into a malignant cell. These discoveries were reviewed in a Grand Rounds in March, 1983 entitled: "Oncogenes: The Wrong Genes In the Wrong Place at the Wrong Time."

Now a new class of cancer-related genes has been discovered, and their implications for medicine are even more profound. In contrast to the viral oncogenes, which are dominant, the new oncogenes are recessive, They cause cancer not by their presence, but by their absence. The recessive oncogenes have already validated the two-hit theory of cancer and they promise to revolutionize our approach to diagnosis, prognosis and treatment of all malignancies.

Like many discoveries in medicine, the recessive oncogenes were excavated by digging among rare diseases, but they were soon implicated in the most common tumors, including colon, breast and lung cancers. In today's Grand Rounds I will review the history of these recessive cancer genes, and then I will outline their implications for the future of medical practice.

Two-Hit Model Of Cancer

The story begins with a school of British epidemiologists who analyzed cancer statistics in the early 1950's. Richard Doll, the father of the two-hit model, noted that certain tumors such as gastric carcinoma increased logarithmically in incidence with age (1). He explained this data by suggesting that the development of cancer requires two independent events, both occurring in the same cell, and both occurring with low frequency. Other epidemiologists, analyzing the same data, concluded that the incidence figures were better explained by a multiple-hit model in which cancer requires as many as six independent events, all occurring in the same cell (2). So by 1970 there was some disagreement as to whether cancer required two genetic hits-or more than two.

Knudson And Retinoblastoma

In 1971 Alfred Knudson, who was then at the M.D. Anderson Hospital in Houston, settled this issue (3) . Knudson had the brilliant idea of using the childhood tumor retinoblastoma as a model. This was a good choice for several reasons. First, retinoblastoma appears almost always by the age of five years. The incidence can be calculated by examining subjects over a very short period thereby avoiding the delayed onset that complicates statistical analysis of other tumors. Second, certain cases of retinoblastoma are transmitted as an autosomal dominant trait. And third, some hereditary cases occur in individuals with a deletion in the long arm of chromosome 13, thereby providing an independent genetic marker of the disease.

Figure 1 depicts a child with retinoblastoma, showing the "white-eye" that is characteristic of this disease. Figure 2 shows the tumor as it appears through the lens. Some retinoblastomas are unilateral, others are bilateral. In many cases there are multiple tumors in one eye.

In a classic paper in PNAS in 1971 (3), Knudson reviewed the data on 48 patients with retinoblastoma seen at the M.D. Anderson Hospital, and he

Fig. 1. A two-year-old girl with retinoblastoma of the left eye (courtesy of Dr. N. Schneider).

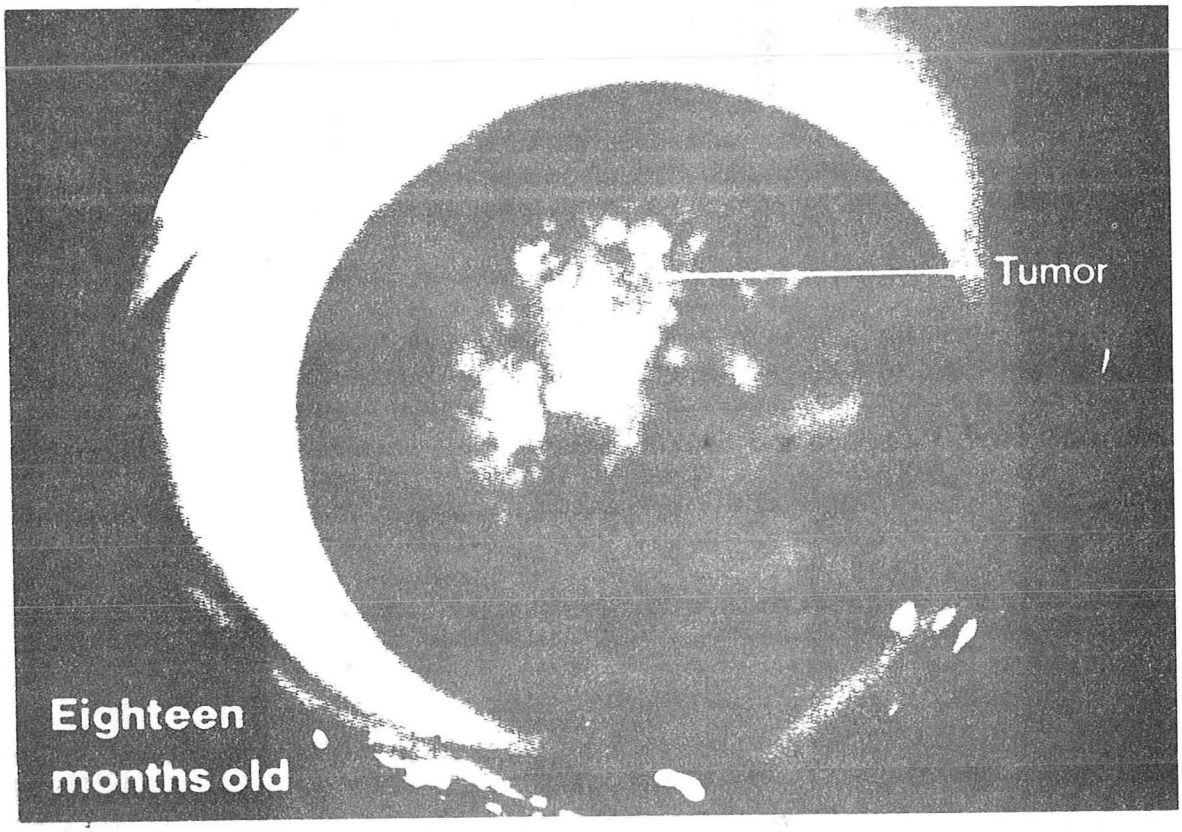


Fig. 2. Large retinoblastoma in an 18 month old child (courtesy of Dr. N. Schneider).

also reviewed two other large series of patients. 35-45% of the cases were hereditary (Fig. 3). These included 25-30% that were bilateral and 10-15% that were unilateral. 55-65% of the tumors were non-hereditary, and all of these tumors were unilateral. Knudson advanced the hypothesis that all of the tumors required two mutant genes. Children with the hereditary form inherited one mutant gene that was defective all retinal cells and they therefore required only one somatic mutation in any retinal cell to produce a cancer. This led to a prediction. If the hereditary tumors required only a single mutation they should be distributed at random in both eyes and the number of tumors in each eye should follow a Poisson distribution. Most eyes should have one tumor, a smaller number should have two tumors, etc. Figure 4 shows the results. In 53% of the eyes there was one tumor, in 26% there were two tumors and in 14% there were three tumors. This percentage closely matched the expected numbers for a Poisson distribution if the mean number of tumors per eye were three. Using an estimate of the number of retinal cells per eye, Knudson was able to calculate a mutation rate for the tumors: 2×10^{-7} per cell per year. If the retinal cells divided every 2 weeks this would give a mutation rate of 1×10^{-6} per cell per generation, which is consistent with the known mutation rate of animal cells under a variety of circumstances.

	Bilateral	Unilateral	Total
Hereditary	25-30%	10-15%	35-45%
Nonhereditary	0	55-65%	55-65%
Total	25-30%	70-75%	100%

Fig. 3. Distribution of Hereditary and Nonhereditary retinoblastomas (3).

*Frequencies of tumors in one eye of bilateral cases for various values of mean number (m) for both eyes**

Tumors, one eye	Expected frequencies (%)				Observed numbers		Observed frequency
	m = 1	m = 2	m = 3	m = 4	14 cases, present series	52 cases, Stallard (6)	66 cases (%)
1	77	59	43	32	7	28	53
2	20	29	33	31	3	14	26
3	3	10	17	21	2	7	14
4		2.5	6	11	1	3	6
5			1.8	4.2	1		1.5

Fig. 4. From (3).

Knudson also plotted the fraction of eyes that did not contain tumors as a function of age (Fig 5). In the individuals with bilateral disease the fraction of eyes that were free of tumors declined exponentially over the first five years of life. This linear exponential decline indicates that only a single random event was necessary in order to produce a tumor. The unilateral cases gave a totally different result. The number of tumors appearing early in life was low. The rate accelerated after about 24 months of age. This finding suggested that the unilateral non-hereditary cases required two mutations. The initial lag was due to the time required to accumulate cells with the first mutation.

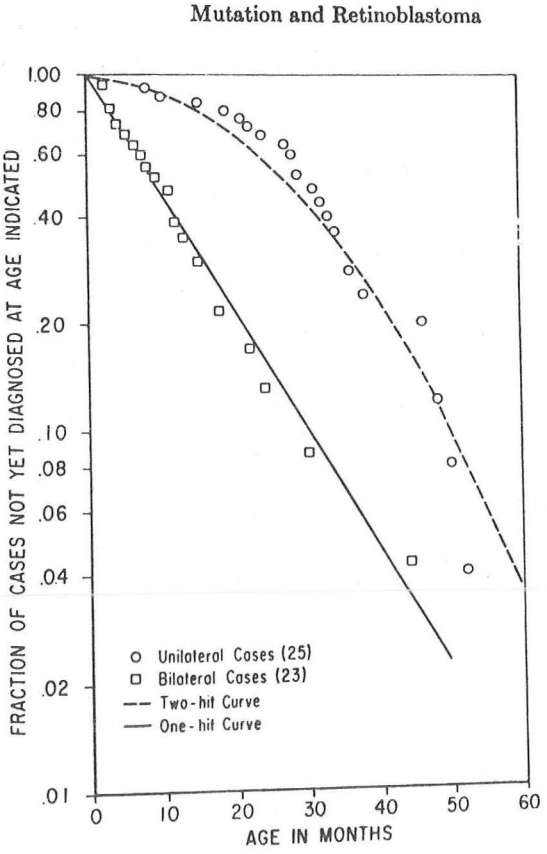


Fig. 5. Exponential decline in tumor-free eyes in bilateral, hereditary, cases of retinoblastoma (solid line) but not in unilateral, sporadic cases (dashed line). From (3).

Knudson concluded from these data that individuals with the hereditary form of retinoblastoma inherited a mutation that affected all of their retinal cells. This mutation presumably occurred on chromosome 13, since some individuals were known to have a deletion on this chromosome. If a second mutation occurred in any of these already-abnormal retinal cells it would produce a tumor. On the other hand, individuals with the sporadic form of retinoblastoma had a requirement for two mutations in order to produce the disease. This sequence is oulined in Fig. 6.

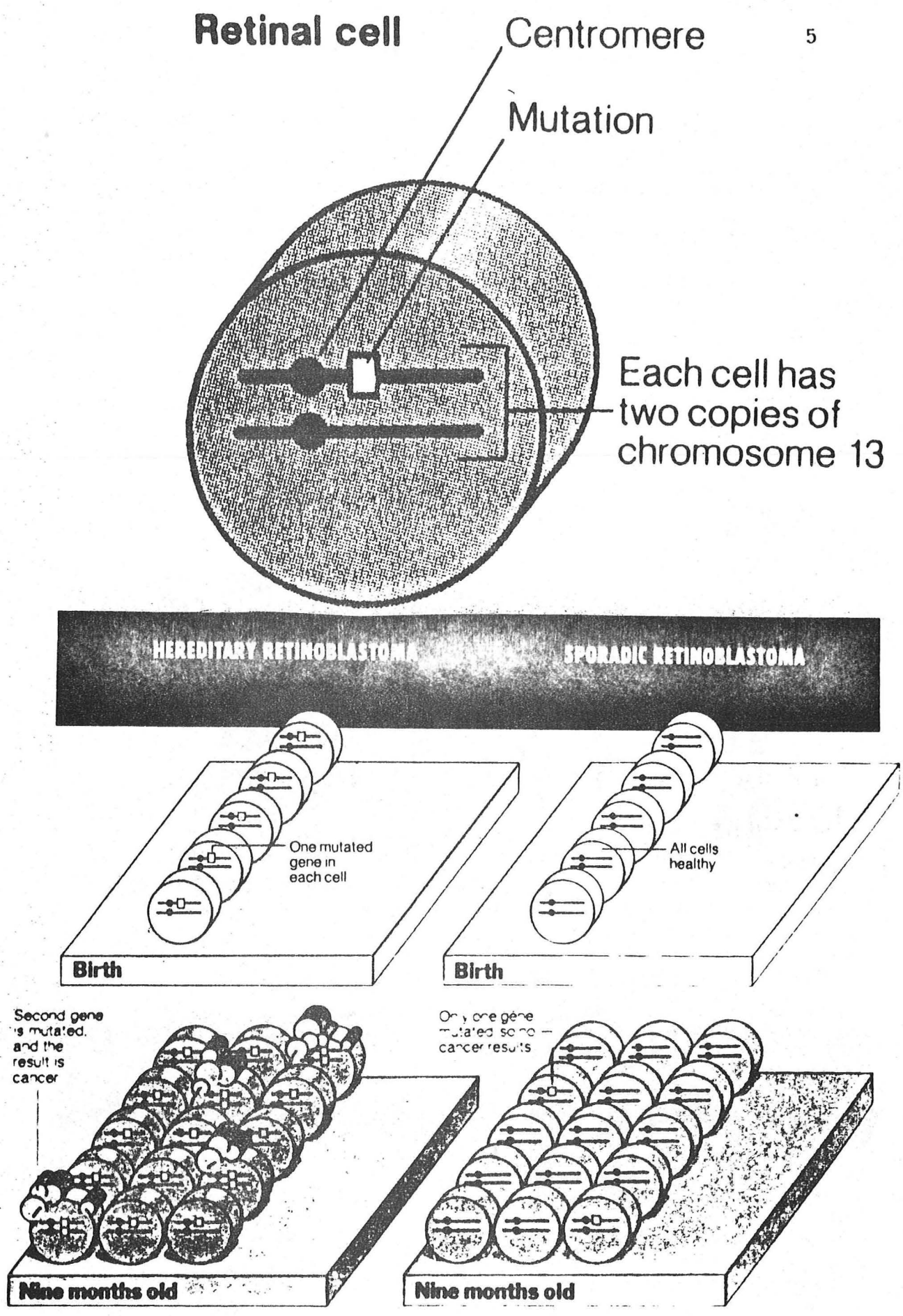


Fig. 6. Sequence of events in development of hereditary and sporadic retinoblastoma. From (32).

Retinoblastoma Gene On Chromosome 13 Band 13q14

The next historical event was the localization of the mutant gene in retinoblastoma to a region of chromosome 13. Many cases of retinoblastoma with visible deletions of the long arm of chromosome 13 had been reported. Affected individuals had multiple congenital anomalies in addition to retinoblastoma. As the techniques for identifying specific bands of chromosomes became more refined, it was possible to identify patients who had much smaller deletions on chromosome 13. Figure 7 is from a 1978 paper by Yunis (5) who observed two children with retinoblastoma who had a very localized deletion on the long arm of chromosome 13 in band 13q14. Yunis reviewed the literature on other reported deletions within chromosome 13 and all of them removed band 13q14 (Fig. 8). Yunis concluded that the retinoblastoma gene was somewhere within this band.

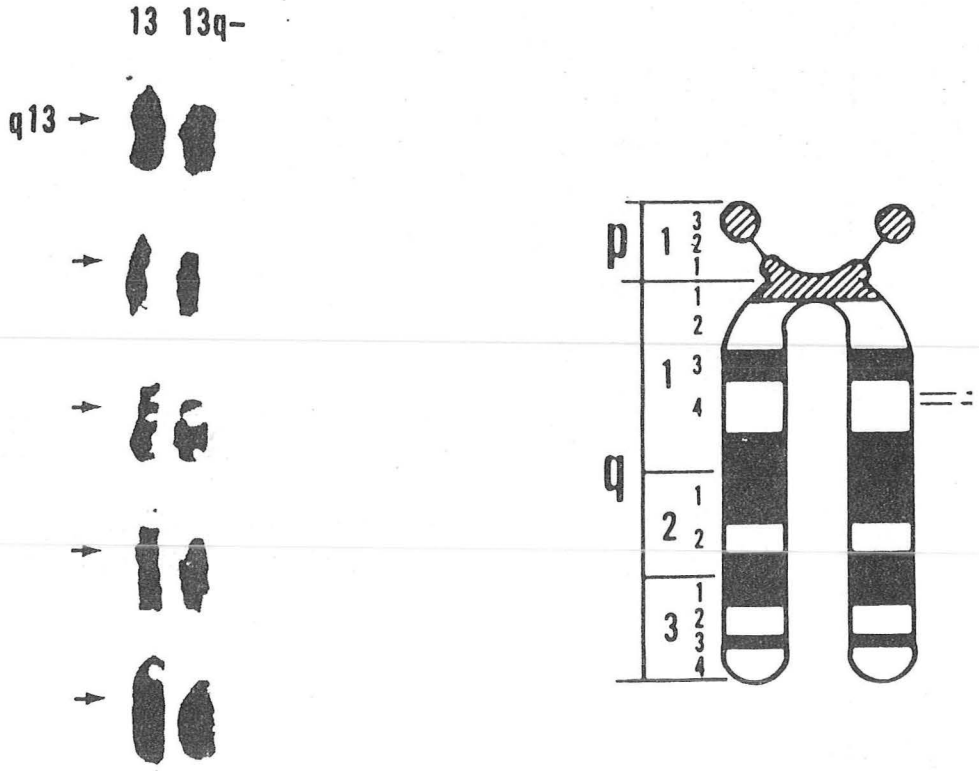


Fig. 7. Chromosome 13 from patient with hereditary retinoblastoma showing deletion on long arm (band 13q14). From (5).

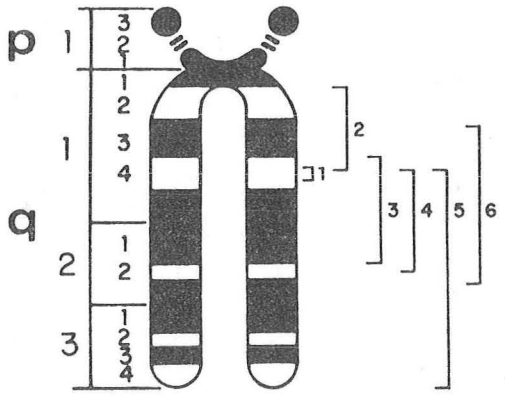


Fig. 8. Chromosome 13 in six cases of hereditary retinoblastoma showing that all cases lack band 13q14. From (5).

The next advance came from the work of a geneticist named Sparkes and a group of ophthalmologists at UCLA who showed that the gene for retinoblastoma is linked to the gene for an enzyme, esterase D (6, 7), that was known to be in the 13q14 band (Fig. 9). One fascinating patient inherited one copy of chromosomes 13 with a deletion that removed the esterase D gene as well as the retinoblastoma gene (7). All of the cells of this patient produced a half-normal amount of esterase D. In the tumor, however, no esterase D was produced at all. Sparkes concluded that the tumor must have somehow become homozygous for the deleted chromosome (7).

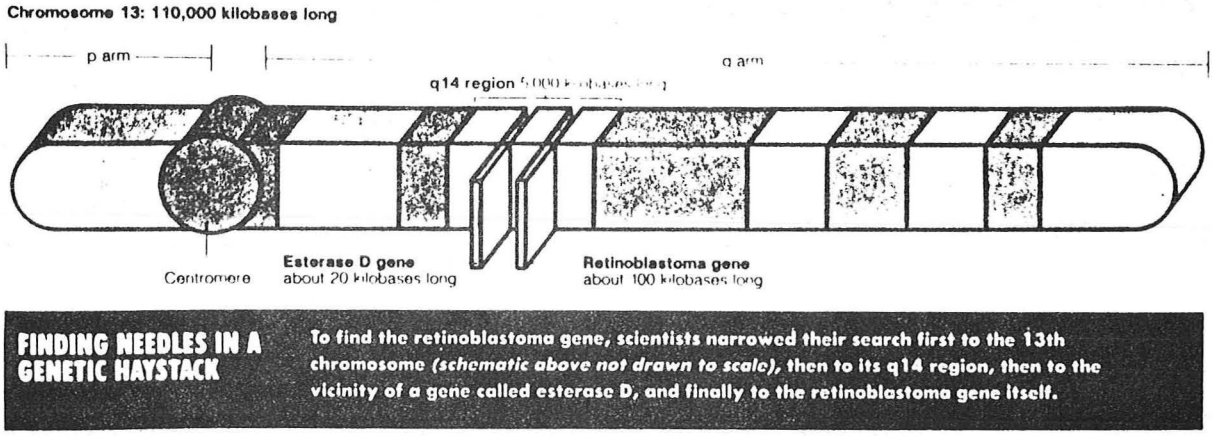


Fig. 9. Location of retinoblastoma gene and gene for esterase D in q14 region of chromosome 13. From (32).

RFLPs and Loss Of Heterozygosity

The next big advance was made in 1984 and it involved the use of restriction fragment length polymorphisms (RFLPs). Cavenee, in collaboration with the UCLA ophthalmologists and with Ray White of the University of Utah, cloned a series of DNA fragments or probes that hybridized to chromosome 13. Several of these probes identified polymorphic loci. When DNA from different individuals is digested with a restriction enzyme, and then hybridized with these probes different sized fragments are obtained from each chromosome depending on whether that chromosome contains sequences that are cleaved by the restriction enzyme (Fig 10 and 11). If the restriction site is present, the DNA is cut into a small fragment. If a restriction site is absent, the DNA gives rise to a large fragment. This technique is most informative when an individual has different restriction sites on each of his two copies of chromosome 13. Each chromosome will then give a characteristic band on the southern gel. This band reveals the presence of that particular chromosome.

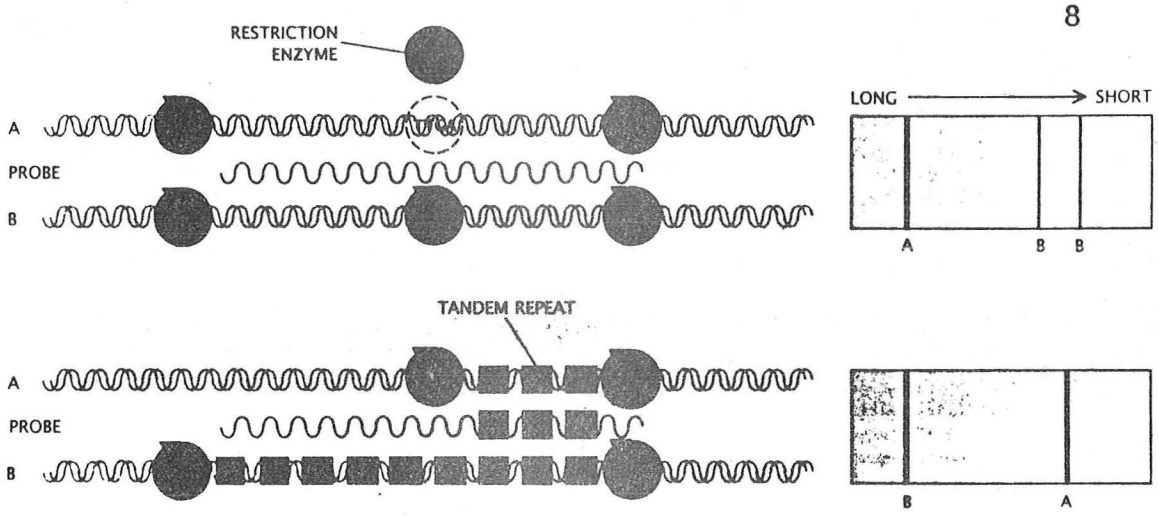


Fig. 10. Two types of restriction fragment length polymorphism. Top: presence or absence of a site for digestion by a restriction enzyme. Bottom: different copy numbers of a short tandem repeat. From (33).

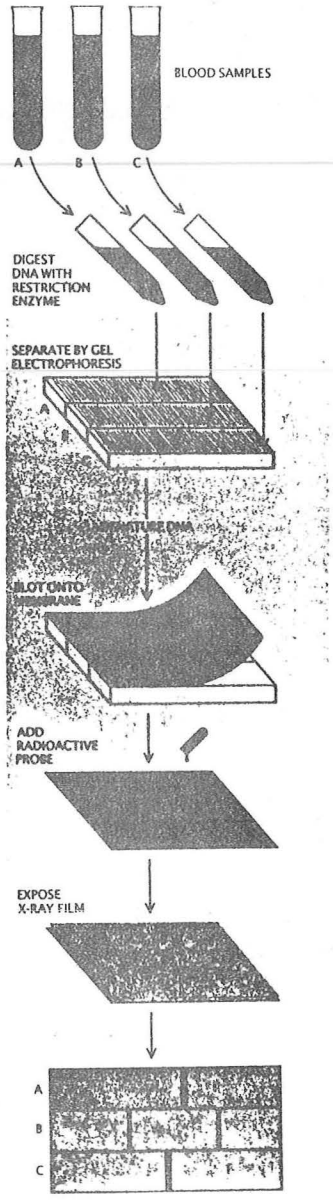


Fig. 11. Technique for study of RFLPs in DNA isolated from blood cells or solid tissues. From (33).

Cavenee and associates isolated DNA from patients with retinoblastoma and performed restriction digests on their somatic cells (usually blood cells) and on DNA isolated from cell lines derived from their tumors (8, 11). Figure 12 shows a typical example. Another example is shown in Figure 14 A. This individual's blood cells were heterozygous for probe 1E8 (2.1).

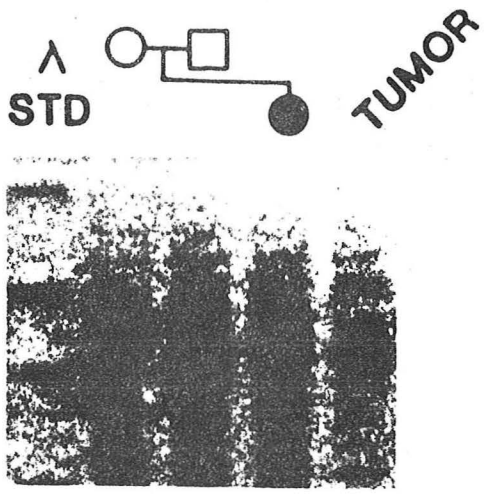


Fig. 12. Loss of heterozygosity for a restriction fragment from band 13q14 in a patient with retinoblastoma. In the tumor the upper band, inherited from the mother, has been lost. From (10).

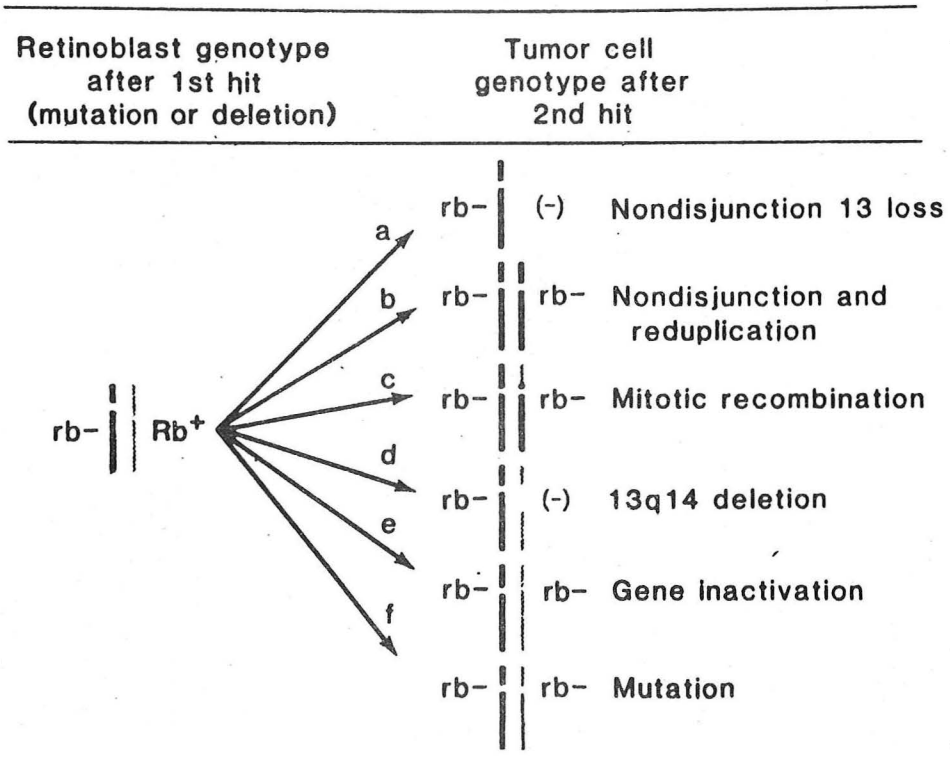


Fig. 13. Six potential mechnisms for loss of heterozygosity at retinoblastoma locus. From (9).

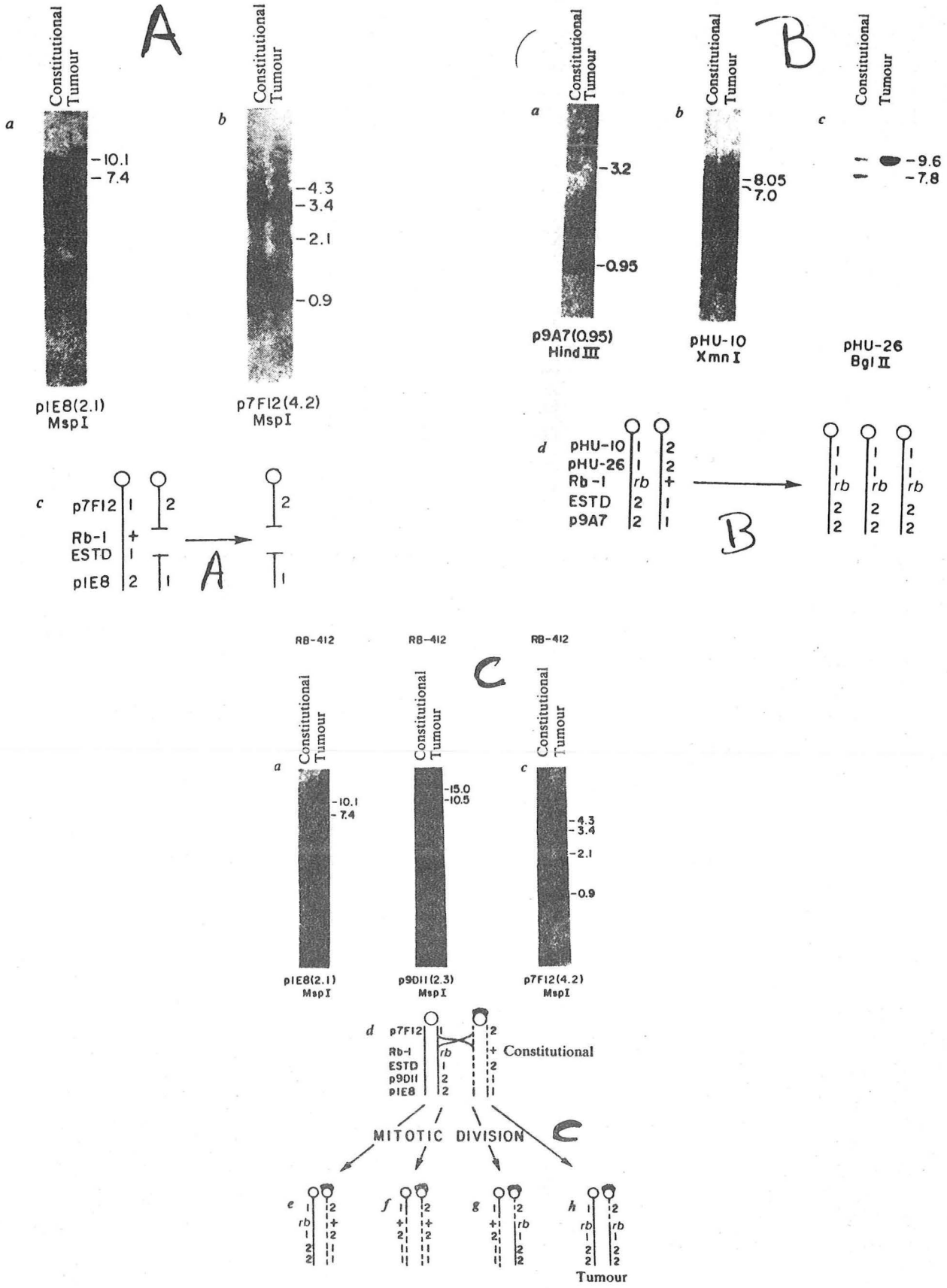


Fig. 14. Retinoblastomas from 3 patients with loss of heterozygosity attributable to nondisjunction (A); nondisjunction followed by triplication (B); and recombination (C). From (8).

When the DNA was digested with the restriction enzyme MSP1 the somatic cells showed two bands, one at 10.1 kilobases and one at 7.4 kilobases. In the tumor, the 7.4 kilobase band was absent. Only the 10.1 kilobase band was seen. A similar phenomenon was observed with another probe P7 F12 which hybridizes to another site on chromosome 13. The constitutional DNA from this subject contained a 4.3 kilobase band that was absent from the tumor. This individual had inherited one normal chromosome 13 and another copy of chromosome 13 with a deletion. The tumor had somehow lost the normal copy of chromosome 13 and contained only a single copy of the deleted chromosome. The heterozygous state in the patient's constitutional cells had been converted to a homozygous state in the tumor. This phenomenon has come to be known as "loss of heterozygosity" and it provides the key to that allows the extension of the two-hit model to all human cancers.

How is a heterozygous state lost in a tumor? Figure 13 shows six potential mechanisms. These include nondisjunction with or without reduplication, mitotic recombination, deletion, gene inactivation and mutation. Figure 14 A, B, and C show examples of loss of heterozygoisty arising via nondisjunction, with reduplication and recombination, respectively. These possibilities are distinguishable by examining the heterozygosity for various polymorphic markers located at different points on the long arm of chromosome 13. Dryja, et. al., (10) studied DNA from 8 patients with retinoblastoma, five of whom had multifocal, presumably hereditary, disease. The other three appeared to have sporadic disease. Loss of heterozygosity for chromosome 13 could be demonstrated in four of these tumors, including one of the unifocal, apparently sporadic cases (10).

In the familial cases in which a parent had a retinoblastoma the tumor tissue from the patient became homozygous for the region of chromosome 13 derived from the chromosome of the affected parent (11), thereby confirming the hypothesis that the loss of heterozygosity initiates the tumor by removing the normal gene inherited from the unaffected parent.

Patients with retinoblastoma are known to have a high incidence of non-retinal tumors that develop after the retinal tumor has been removed (12). The most frequent secondary tumor is osteogenic sarcoma and its incidence of these tumors is markedly increased by radiation treatment of the retinoblastoma. Dryja and co-workers (13) showed that in three of fifteen patients the osteogenic sarcomas showed homozygosity for all loci on chromosome 13. In one of these individuals constitutional DNA was available, and it showed heterozygosity at several loci on chromosome 13. Thus, this osteogenic sarcoma had lost its heterozygosity, just as did the retinoblastoma. It is likely that the other two tumors had also lost heterozygosity, but no constitutional DNA was available for study. Interestingly, chromosome 13 from three patients with osteogenic sarcoma without retinoblastoma was also homozygous at all chromosome 13 loci, suggesting that loss of heterozygosity at 13q14 is important in sporadic osteogenic sarcoma as well as in the hereditary type.

Cloning of Retinoblastoma Gene

After the locus of the retinoblastoma gene on 13q14 became known, Friend and co-workers in Boston used techniques of chromosome-walking and interspecies hybridization to isolate the gene for retinoblastoma (14) (See Fig. 15). The UCLA group, using similar techniques isolated the same gene, as well as a cDNA (15, 16). The gene was identified by its ability to hybridize to DNA from multiple animal species, indicating that it encoded a protein and was therefore conserved in sequence (Fig. 16). The gene encodes a protein

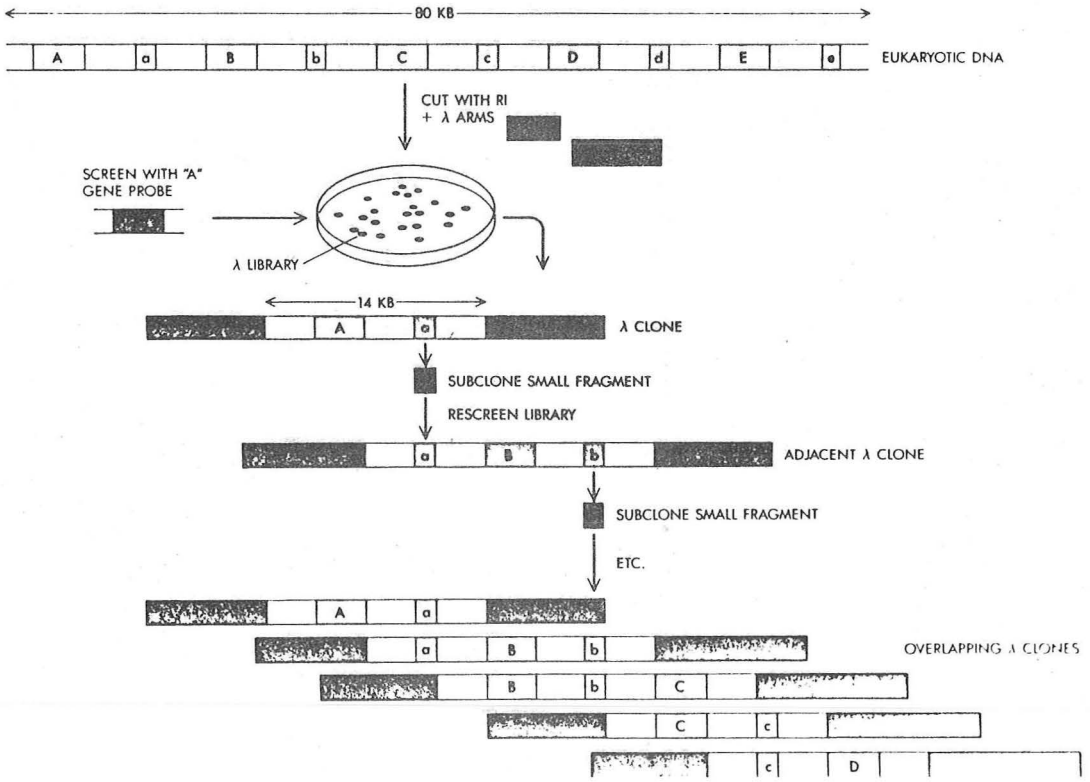


Fig. 15. Technique of chromosome walking that was used to clone the retinoblastoma gene.

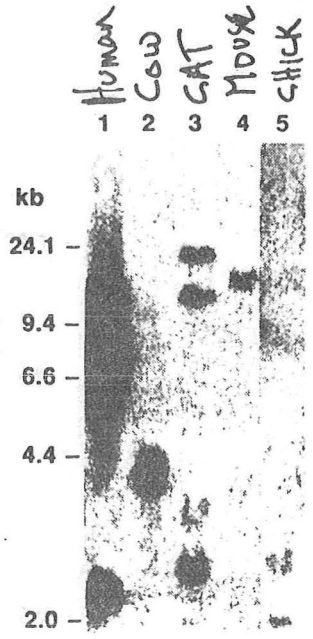


Fig. 16. Conservation of sequence of retinoblastoma gene in animal species as determined by blot hybridization of the human gene to restriction digests of DNA from various animals. From (15).

of 816 amino acids (94,000 daltons) that lacks a signal sequence and a transmembrane region, implying that it is a cytosolic or nuclear protein. It is not an obvious DNA-binding protein and it has no homology to any other protein. Interestingly, the mRNA is produced in a wide variety of tissues in addition to fetal retina. These include brain, kidney, ovary and placenta (Fig. 17). Thus, the retinoblastoma gene must function in a wide variety of tissues, yet its deletion leads to tumors primarily in the retina and in the bones. Perhaps other tissues have backup genes that substitute for the retinoblastoma gene.

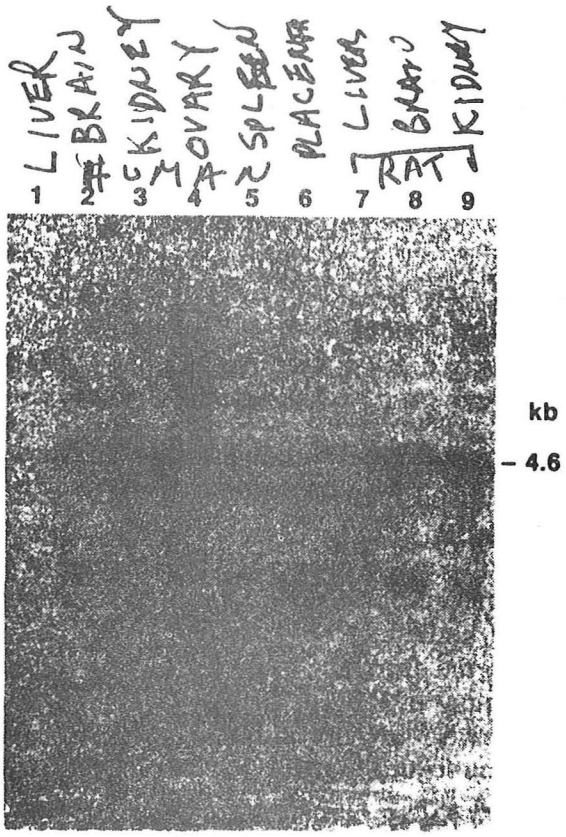


Fig. 17. Expression of messenger RNA from retinoblastoma gene in various tissues of humans (lanes 1-6) and rats (lanes 7-9) as determined by blot hybridization with cloned human retinoblastoma gene. From (15).

The availability of closely linked markers for the retinoblastoma gene has allowed early diagnosis of retinoblastoma in children who are known to be at risk (17, 18).

Loss of Heterozygosity in Other Embryonal Tumors

The sequence of events in retinoblastoma has been reproduced in three other embryonal tumors, Wilms' tumor, hepatoblastoma and rhabdomyosarcoma (19, 20). Each of these tumors shows a high frequency of loss of heterozygosity for loci on the short arm of chromosome 11.

Colon Carcinoma

Loss of heterozygosity has recently been demonstrated in the most common cancers of humans, including colon carcinomas. The story begins in 1986 with a brief clinical report from the Roswell Park Memorial Institute (22).

The authors observed an individual with multiple congenital anomalies, mental retardation, and Gardner syndrome. Karyotype analysis demonstrated a small deletion on the long-arm of chromosome 5. Gardner syndrome and familial polyposis of the colon are closely related syndromes that are thought to be caused by mutations at the same genetic locus. Affected patients develop multiple adenomatous polyps of the colon at puberty, and almost always develop malignant degeneration of these polyps later in life.

Stimulated by the finding of a chromosome 5 deletion, Bodmer and his colleagues in England obtained DNA samples from several families with autosomal dominant familial FAP and examined their DNA with probes that revealed restriction fragment length polymorphism on chromosome 5 (23). One of the chromosome 5 probes (called C11P11) detected a polymorphism that segregated in six families together with FAP. Figure 18 shows one family in which the grandmother, who had died of FAP, is inferred to have had two different alleles at this locus, one giving rise to a 4.4 kb band, and the other giving rise to a 3.9 kb band after digestion with a restriction enzyme. She had five offspring. The two offspring that inherited her 3.9 kb band both developed FAP. The three offspring who inherited her 4.4 kb band were free of disease. The affected offspring passed the disease to their children who inherited the 3.9 kb band. The authors performed similar analyses on a total of six families in whom the heterozygosity was informative, and they showed that FAP was closely linked to this chromosome 5 probe. The lod score for recombination was 3.26 at a recombination fraction of zero (Fig. 19). In fact, no recombinations were observed between this locus and FAP. These findings indicate that the C11-P11 probe is located physically very close to the gene responsible for FAP on chromosome 5.

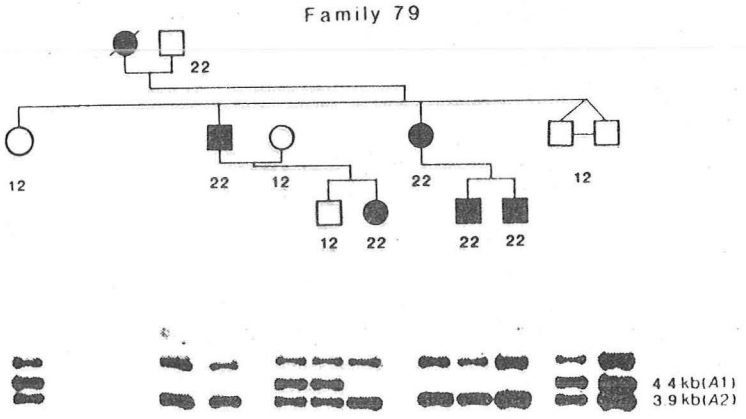


Fig. 18. Family 79 with FAP. The affected grandmother (deceased) is inferred to possess one chromosome 5 with a 4.4 kb restriction fragment and another with a 3.9 kb fragment. All offspring who inherited the chromosome with the 4.4 kb fragment were unaffected. All offspring who inherited the chromosome with the 3.9 kb band fragment had FAP. From (23).

Lod scores from two-point analyses for linkage between C11p11, L1.4 and FAP

	Recombination fraction (θ)				
Marker loci	0.0	0.10	0.20	0.30	0.40
C11p11-FAP	3.26	2.56	1.88	1.15	0.44
L1.4-FAP	−2.50	0.78	0.66	0.37	0.12

Fig. 19. From (23).

The next step taken by these workers was even more startling (24). Based on Knudson's hypothesis of the relation between hereditary and sporadic forms of cancer, they reasoned that even in nonheriditary cases of colon carcinoma there should be a loss of heterozygosity on chromosome 5 attributable to two independent mutational events. They could not use the C11-P11 probe for this purpose because the incidence of heterozygosity in the general population was not high enough. However, they were able to identify other probes on chromosome 5, closely linked to C11-P11, which showed a high frequency of heterozygosity in the population. These probes identified reagents of the chromosome that contained a variable number of tandem repeat sequences (VNTRs). Two of the probes were informative in fifteen patients. The tumors in six of these fifteen patients showed a loss of heterozygosity for one or the other probe, or both (Fig. 20). They concluded that as many as 40% of colon cancers had lost heterozygosity for a region of chromosome 5. Specificity of this finding was indicated by the failure to find loss of heterozygosity with probes that hybridized with six other chromosomes.

Summary of cases where both probes are informative

λMS8	L1.4	λMS8	L1.4	λMS8	L1.4	λMS8	L1.4	
no loss	no loss	no loss	loss	loss	no loss	loss	loss	Total
9		1		1		4		15

Fig. 20. Loss of heterozygosity for two probes on chromosome 5 in 15 patients whose constitutional DNA was heterozygous for both probes. From (24).

A similar incidence of loss of heterozygosity on chromosome 5 was reported just last month in a paper from Japan in patients with sporadic colon carcinoma or carcinomas associated with FAP (25). These authors also found a high incidence of loss of heterozygosity on chromosome 17. In still another study 76% of colon carcinomas were shown to have a loss of heterozygosity on chromosome 17 (26) (See Fig. 21). Again, both familial and sporatic cases showed the same findings. These authors did not study probes from chromosome 5.

Based on the above findings, the precise pattern of heterozygosity loss in colon carcinoma is not yet firmly established. Clearly, the locus for FAP is on chromosome 5, and loss of heterozygosity at this locus is probably responsible for the majority of hereditary colon cancers in these patients.

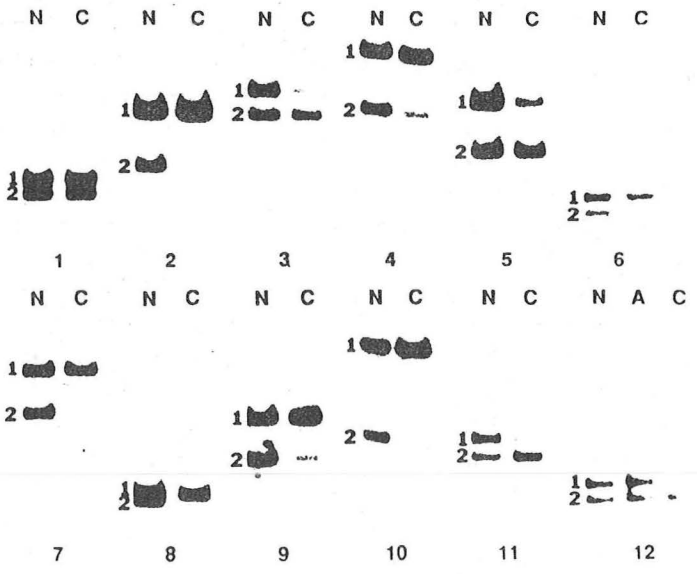


Fig. 21. Loss of heterozygosity for chromosome 17 markers in 75% of patients with colon carcinomas. From (26).

Chromosomes 17 and 22 may also contain recessive oncogenes that are involved in colon cancer. Importantly, none of these chromosomes showed loss of heterozygosity in nonmalignant adenomas. Thus, the loss of heterozygosity seems to occur at the time that the adenoma converts to a carcinoma.

Breast Carcinoma

In ductal carcinoma of the breast four of ten tumors (40%) were noted to have a loss of heterozygosity for multiple loci on chromosome 13 (27) (See Fig. 22). The patients included young women and two males. Chromosome 13 is the same chromosome that is involved in retinoblastoma. Insufficient data are available however, to determine whether the affected gene is the same one that is involved in retinoblastoma. In order to answer this question it will be necessary to perform fine mapping studies on patients with breast cancer who have lost small parts of chromosome 13 owing to deletion or recombination.

Genotypes in breast tumor DNA at loci on chromosome 13

Case no.	Locus (enzyme) D13S6 (*Xmn* I)	D13S1 (*Msp* I)	D13S1 (*Taq* I)	D13S2 (*Msp* I)	D13S2 (*Taq* I)	D13S4 (*Msp* I)	D13S5 (*Hin*dIII)	D13S5 (*Eco*RI)	D13S7 (*Bgl* II)	D13S3 (*Msp* I)	D13S3 (*Hin*dIII)
					Ductal carcinoma						
BC1		1, 2	1, 2	—	—	—	—			1, 2	
BC2		—	—	—	—	—	—			1, 2	
BC4		1, 2	—	—	1, 2	—	—			—	—
BC6	—	—	*1*	*1*	—	*1*	—			*2*	*1*
BC11	*2*	—	—	—	—	—	*1*	*1*	*1*	—	—
BC14	—	—	*1*	*1*	—	*2*	—			—	—
BC18		—	—	—	—	1, 2				1, 2	
BC20	—	—	1, 2	—	—	—	—		—	—	—
BC21	—	—	—	—	1, 2	1, 2	1, 2	—	—	—	
BC27	*2*	*2*	*2*	—	*1*	—	—		—	*1*	*1*
					Comedocarcinoma						
BC3	—	—	—	1, 2	—	—	1, 2	—	—	1, 2	—
BC15	1, 2	—	—	—	—	—	—	—	—	—	—
					Medullary carcinoma						
BC24	1, 2	1, 2	1, 2	1, 2	—	—	1, 2		—	1, 2	1, 2
					Juvenile secretory carcinoma						
BC29		—	1, 2	—	—	1, 2	—		1, 2		

Numbers indicate the restriction fragment length alleles present in tumor tissue at loci that were constitutionally heterozygous. Italicized numbers indicate loss of a constitutional allele. — indicates constitutional homozygosity.

Fig. 22. From (27).

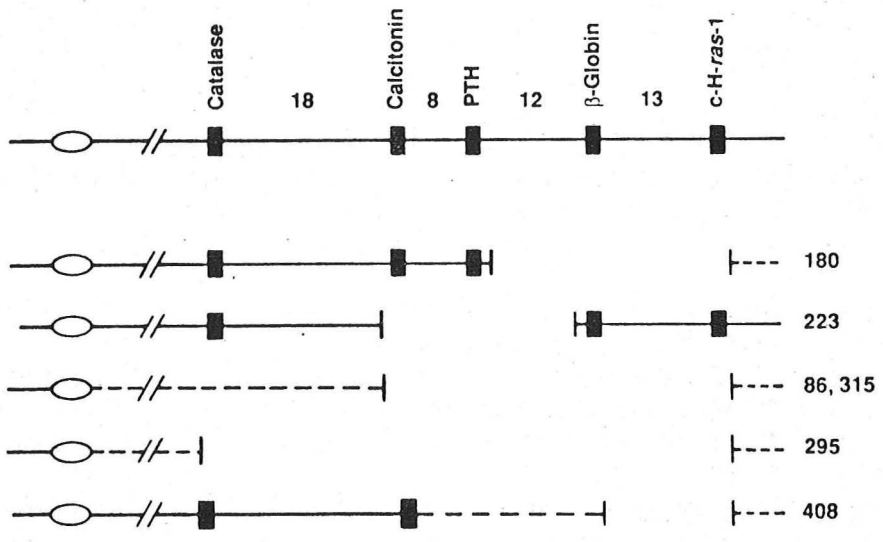


Fig. 23. Deletion on chromosome 11 in breast cancer patients as inferred from loss of heterozygosity for various RFLPs. From (28).

In another recent study of 56 patients with breast cancer, 20% were noted to have a loss of heterozygosity on chromosome 11 (28). The loss of heterozygosity involved regions of the gene near the locus for the genes for calcitonin, beta-globin and parathyroid hormone (Fig. 23). The incidence of 11p deletions was related to the state of differentiation of the tumor (Fig. 24). In the most differentiated tumors (class 1) there were no deletions on chromosome 11. In the most advanced cases, 71% of the tumors showed such deletions. The incidence of deletions was also higher in tumors that failed to express estrogen receptors or progesterone receptors, a set of findings that is known to be associated with a more malignant course. These findings suggest that the loss of heterozygosity on chromosome 11 is not a primary event in breast cancer but one that occurs after the tumor has initiated, and one that may lead to a more malignant state.

Correlation between chromosome 11p deletion and clinico-histopathological parameters.

Parameter*	Total tumor population [*n* (%)]	Tumors with deletions on chromosome 11 [*n* (%)]	Tumors without deletions [*n* (%)]	χ^2 analysis
I	10 (14)	0 (0)	10 (18)	
II	34 (47)	5 (29)	29 (53)	
III	28 (39)	12 (71)	16 (29)	$P < 0.006$
ER^+	55 (66)	8 (44)	47 (72)	$P < 0.025$
ER^-	28 (34)	10 (56)	18 (28)	$P < 0.0025$
PR^+	49 (59)	5 (28)	44 (68)	
PR^-	34 (44)	13 (72)	21 (32)	
M^-	66 (79)	11 (61)	55 (83)	$P < 0.05$
M^+	18 (21)	7 (39)	11 (17)	

*I, II, and III represent histopathologic grades; ER, estrogen receptor; PR, progresterone receptor; M^- and M^+, the absence or presence (respectively) of distal metastasis or local reoccurrence.

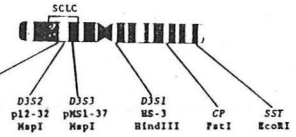
Fig. 24. From (28).

The exciting aspect of the findings on breast carcinoma relate to the fact that about 5% of cases are transmitted in families as an autosomal dominant trait. It should now be possible to analyze these families with probes from chromosomes 13 and 11 to determine whether affected women inherit a single mutant gene on one of these chromosomes that predisposes to breast cancer. If the dominant gene can be shown to segregate with one of these chromosomes, and if this chromosome then loses heterozygosity in the tumor, the situation would be exactly analogous to that in retinoblastoma in which the hereditary patients start with one mutation, whereas the sporadic cases require two mutations. It should also be possible to isolate the responsible gene in the same way that the gene for retinoblastoma was isolated. Finally, it should be possible to provide accurate genetic counseling to women from affected families. Such women can be followed carefully for the first sign of malignancy. Some of them might even request prophylactic mastectomies.

Lung Carcinoma

An exciting, but still somewhat confusing story has begun to unfold with lung cancer. Three papers on this subject appeared in the last three months of 1987. Minna and co-workers (29) reported that nine out of nine patients (100%) with small cell lung cancer had loss of heterozygosity for the short arm of chromosome 3 (Fig. 25). In several of these patients an entire copy of chromosome 3 was deleted owing to nondisjunction. In others, the loss of heterozygosity was restricted to a small area on the short arm of chromosome 3. Markers on other chromosomes showed occasional loss of heterozygosity but there was no consistent pattern as there was with chromosome 3p.

Chromosome 3 markers in SCLC



Group 1:						
H209BL	1,2	1,2	1,2	1,1	-	1,2
H209	1	1	1	1,1	-	1,2
N-1	1,2	1,1	1,1	1,2	1,2	1,1
SCLC-1	2	1,1	1,1	1,2	1,2	1,1
N-2	2,2	1,2	1,1	1,2	1,2	1,2
SCLC-2	2	2	1,1	1,2	1,2	1,2
N-3	1,2	1,1	1,2	1,2	1,2	1,1
SCLC-3	2	1	1	1,2	1,2	1,1
N-5	1,2	1,2	1,2	1,1	1,2	1,2
SCLC-5	2	1	1	1,1	1,2	1,2
N-6	2,2	1,2	1,2	1,2	1,1	1,2
SCLC-6	2	1	2	1,2	1,1	1,2
N-7	1,1	1,2	1,1	1,2	1,1	1,1
SCLC-7	1	2	1,1	1,2	1,1	1,1
Group 2:						
H128BL	2,2	1,2	1,2	1,2	-	1,1
H128	2	1	1	2	-	1
N-4	1,2	1,2	1,2	1,2	1,1	1,1
SCLC-4	1	2	1	1	1	1
Other lung cancers:						
N	2,2	1,2	1,1	1,2	2,2	1,1
Large cell	2,2	1,2	1,1	1,2	2,2	1,1
N	1,2	2,2	1,1	1,1	1,2	1,1
Adenocarcinoma	1,2	2,2	1,1	1,1	1,2	1,1

Fig. 25. From (29).

A 100% loss of heterozygosity for 3p was also found in seven small cell lung carcinomas from Japan (30). These authors also found a high incidence of loss of heterozygosity on 13q (91%) and 17p (100%). These authors also found a high incidence of loss of heterozygosity for chromosome 3p in adenocarcinoma of the lung.

Most recently a group of European investigators also found loss of heterozygosity for chromosome 3p in 100% of lung cancers (31). The samples included five cases of small cell lung cancer and 16 cases of squamous cell carcinoma. Similar findings were observed with adenocarcinoma of the lung. Considered together, the data on lung cancer provides strong evidence for a recessive oncogene on chromosome 3p that may be implicated in all forms of lung cancer. It is likely that other recessive oncogenes on other chromosomes may be involved in certain cases as well.

. Histological types of tumors with loss of heterozygosity at loci on chromosomes 3p, 13q, and 17p

Type of tumor	Loss of heterozygosity on chromosomes		
	3p	13q	17p
SCC	7/7	10/11	5/5
AdC	5/6	4/15	2/7
SqC	0/1	4/11	1/4
LCC	0/1	1/3	0/0
ASC	0/0	1/2	0/1
Total	12/15	20/42	8/17

Fig. 26. From (30).

Conclusions and Implications

We are clearly entering a new era in the clinical assessment of solid tumors. The breakthrough that emerged from the work on retinoblastoma has opened a pandora's box full of insights into the genetic basis of common tumors. The results are appearing even faster than one can read them. It is clear that loss of both copies of certain genes plays a major role in the genesis of many human cancers.

One can invision that the next few years will see a massive increase in the amount of such data. Loss of heterozygosity on various chromosomes will be determined for many forms of cancer. Different cases of the same tumor may show loss of heterozygosity for different chromosomal regions. The first fallout from this type of work has already appeared. It is possible to predict which individuals in a pedigree will develop retinoblastoma. It is also now possible to predict which individuals in a pedigree with familial adenomadous polyposis will develop carcinomas. In a short time we will be able to predict which women in affected families will be at risk for developing breast cancer.

But will these predictions apply only to the dominantly inherited cancer syndromes that are now recognized? I doubt it. I believe that this work will reveal new syndromes in which loss of one pair of genes can lead to the development of several different types of tumor. We already know one example. In retinoblastoma families affected patients can have osteosarcoma as well as retinoblastoma. Is it possible that many individuals inherit a single copy of a mutant recessive oncogene that can predispose to any one of a variety of common tumors? Such families would appear to have a high incidence of cancer, but without aggregation as a specific type. Indeed, a syndrome called the "cancer family syndrome" has been reported in which affected relatives can have one of a group of common cancers. Up to now, however, our ability to study such families has been limited because we can only diagnose them after the onset of a tumor. It seems likely that we will be able to find linked markers that segregate with tumors in these families, and we can then use these probes to identify the mutant genes. Once that gene has been identified, it may turn out that many people in the population have mutations in that gene, and therefore we should be able to identify many individuals at risk for common forms of cancer.

At a fundamental level, the new insights into the recessive anti-oncogenes have exposed a new class of proteins that function to prevent the malignant state. There are many speculations as to how these proteins might work. They might be gene regulators that turn other genes on or off, thus causing cells to become growth arrested. It seems likely that there will be a family of these genes, and once they are isolated it should be possible to work out their roles in differentiation, perhaps by finding the homologues in lower species such as drosophila when experimentation is possible.

Again, clinical medicine has provided the stimulus that many lead to a great increase in our knowledge of the ways in which cellular differentiation occurs. There is much more to be learned about the recessive oncogenes, and the next few years should prove exciting.

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