development of retinoblastoma in at least these two forms of the disease. This interpretation is also consistent with the theory of Knudson (*6*) who believes the manifestation of retinoblastoma depends upon the presence of at least two mutations. However, these results speak against the contention of Matsunaga (*7*) who has argued that the major retinoblastoma gene is probably not located on 13q.

While these studies do not give further insight into the normal function of the retinoblastoma gene, they may, however, assist in the eventual isolation of this gene and the identification of its function.

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Patient with 13 Chromosome Deletion: Evidence That the Retinoblastoma Gene Is a Recessive Cancer Gene

Abstract. *Although a constitutional chromosomal deletion including 13q14 has been found to date in all retinoblastoma patients whose esterase D activity is 50 percent of normal, one female patient has been found who has 50 percent esterase D activity in all normal cells examined but no deletion of 13q14 at the 550-band level. Therefore, she has the smallest constitutional chromosomal deletion within 13q14 that is associated with susceptibility to retinoblastoma. Two stem lines were identified in a retinoblastoma from this patient, and each one had a missing 13 chromosome. No detectable esterase D activity was found in the tumor, indicating that the normal nondeleted 13 chromosome was lost in both stem lines. Thus the data from this patient not only show that there is a total loss of genetic information at the location of the retinoblastoma gene within the tumor, but also imply that recessive genes may play an important role in the development of certain human tumors including retinoblastoma.*

A small percentage of patients with hereditary retinoblastoma have an associated constitutional deletion of chromosomal region 13q14. Although retinoblastoma is a rare tumor, it is important because the genetic susceptibility to retinoblastoma may provide a unique opportunity to examine the molecular basis for the development of a specific human tumor.

Esterase D (ESD), an enzyme of unknown function that is found in most tissues, has also been assigned to chromosomal region 13q14 by deletion mapping; that is, the gene producing it occurs at the same location as the retinoblastoma gene (*1*, *2*). The activity of this enzyme depends on the "dose" of the gene (*1*). Thus, the tissues of individuals with a deletion of one 13 chromosome, which includes q14, show only 50 percent of the ESD activity found in normal individuals. Esterase D is also an electrophoretic polymorphism with the two common alleles being types 1 and 2. The close proximity of the loci for the enzyme and deletion retinoblastoma should enable one to identify individuals with a deletion of 13q14 beyond the resolution of chromosomal banding. In addition, examination of the tumor chromosomal patterns and ESD levels within a patient with retinoblastoma should provide a clue to the mechanism of human tumorigenesis. In this report we describe data from such a patient indicating that the retinoblastoma gene is a recessive gene at the cellular level.

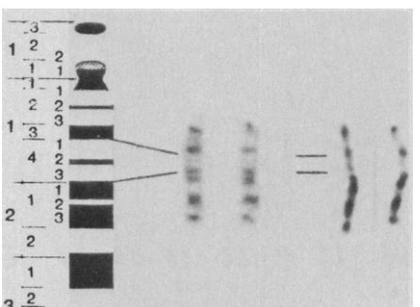

Fig. 1. High-resolution G banding showing the normal banding pattern of 13q14. The diagram of the 13 chromosome at the left indicates segments 14.1, 14.2, and 14.3. All three bands are present in the two 13 chromosomes shown from representative metaphases of peripheral blood lymphocytes (center) and a lymphoblastoid line (right). Thus, there is no evidence of any chromosomal deletion of 13q14 in this patient at approximately the 550-band level (*3*).

The patient is a 3-year-old female who was admitted to the Childrens Hospital of Los Angeles with bilateral retinoblastoma. Except for the presence of the tumor, no other physical or developmental abnormalities previously associated with the 13q14 syndrome (*2*) were found. The ESD activity in her red blood cells was 50 percent of normal (Table 1). Since both of her parents had normal ESD activity, the probability that she inherited a null allele for the enzyme was extremely small. Evaluation of a large number of additional markers gave no evidence of nonpaternity.

The probability of separate mutations occurring simultaneously in the ESD and retinoblastoma genes is remote; therefore, we suspected that the patient had a deletion within band 13q14. However, an examination of more than 40 early metaphase spreads from her peripheral blood lymphocytes showed that at the 550-band level (*3*), all three bands of 13q14 were present (Fig. 1). Since ESD analysis was done on the red blood cells, whereas the chromosomal analysis was performed on peripheral lymphocytes, the possibility of a mosaicism for 13q14 deletion could not be excluded. Therefore, we carried out the ESD assay and

the chromosomal analysis on skin fibroblasts and on a lymphoblastoid line established by one of us (C.S.). Again, the enzyme activity was approximately 50 percent of the normal values in both the fibroblasts and the lymphoblastoid line (Table 1), and at the 550-band level no deletion of 13q14 was found (Fig. 1). Thus, the patient has the smallest deletion within 13q14 yet reported.

When the right eye was removed a portion of the retinoblastoma was sent for chromosomal analysis. Direct chromosomal preparations from the primary tumor revealed two distinct stem lines that were represented approximately equally in the ten karyotypes analyzed. The first stem line had a modal karyotype of 49, X, +2, +7, −13, +15, −16, −17, −18, −21, $+M_1$, $+M_2$, $+M_3$, plus three other unidentifiable chromosomes (Fig. 2A). None of the abnormal chromosomes appeared to contain a portion of chromosome 13. The marker chromosomes M_1, M_2, and M_3 were present in every karyotype from this stem line. In contrast, the second stem line had a modal karyotype of 49, XX, −13, +19, +20, +22, $+M_4$. Since there were numerous differences in the karyotypic patterns between the two chromosomal lines, including unique marker chromosomes, they could not have resulted from clonal evolution of only one transformed cell. Rather, they must have been derived from two separate transformation events. The only consistent chromosomal deviation from the normal diploid pattern found in both stem lines was a loss of a 13 chromosome.

Since the deleted and nondeleted 13 chromosome appear normal cytogenetically, the ESD activity and the electrophoretic pattern provided the only way to determine which of the two chromosomes (the deleted or nondeleted) was lost in the tumor. When this study was conducted no ESD activity was found in either the original tumor or the tumor passaged intraocularly in the nude mouse (Table 1). The passaged tumor also had a missing 13 chromosome in all the karyotypes analyzed but only one of the two stem lines found in the original tumor was present (Fig. 2B). These results, therefore, indicate that the deleted chromosome 13 was retained, whereas the normal chromosome 13 was lost in both stem lines.

That the normal 13 chromosome was lost in both stem lines is of considerable theoretical importance, because at the tumor level there was a total loss of genetic information within 13q14, the locus for the retinoblastoma gene. This complete loss of the gene in the tumor would indicate that the gene for susceptibility to retinoblastoma is probably a recessive gene at the cellular level, a possibility initially proposed by both Comings and Knudsen and subsequently extended by us (*4*).

Since we have recently shown that the hereditary nondeletion form of retinoblastoma is also located in 13q14 (*5*), we maintain that there is only one retinoblastoma gene and that this gene represents a prototype of a class of human cancer genes characterized by a loss of genetic information at the constitutional or tumor level. This class of cancer genes includes Wilms's tumor (*6*), neuroblastoma (*7*), small cell carcinoma of the lung (*8*), and renal cell carcinoma (*9*).

These findings should be contrasted with a second class of potential human "cancer genes" which may function by gene activation rather than by gene inactivation or loss. This second class of cancer genes could include the human cellular oncogenes isolated from human tumor DNA by transfection into nonhuman cells. Although their role in human tumorigenesis has yet to be determined (*10*), they may also have a role in the etiology of certain human cancers.

Since the retinoblastoma gene located in 13q14 governs susceptibility to a human cancer, the potential importance of the patient we describe in helping us to understand the nature of human cancer genes cannot be overemphasized for the following reasons. (i) She has, to date, the smallest constitutional deletion within 13q14. (ii) She is the first individual in which it has been possible to determine that there is a total loss of genetic material at a chromosomal locus known to contain a cancer gene for that specific human malignancy. This finding in turn suggests that the retinoblastoma cancer gene is recessive and that both alleles must be lost for tumors to develop. (iii)

Table 1. Esterase D activity in various cell types obtained from the patient compared to ESD activity in similar cell types from control sources. The ESD activity is expressed as 10^{-7} moles of methylumbelliferyl acetate metabolized per hour per gram of hemoglobin (*11*), whereas the activity in fibroblasts, lymphoblastoid cells, and tumor cells were based on grams of protein rather than hemoglobin.

Red blood cells		Fibroblast cells		Lymphoblastoid lines		Tumor cells	
Patient	Control	Patient	Control	Patient	Control	Patient	Control
38.9	67.4*	34.2	58.8	21.2	39.4	No activity†	67.4‡
38.8	76.9*	42.6	67.9	33.4	54.2	No activity¶	76.9‡

*Parental blood samples. †The tumor cells examined were from the original tumor. ‡The parent's red blood cells were examined in the same assay as controls rather than tumor cells. ¶The tumor cells used were pooled, second-passage intraocular tumors grown in the nude mouse. Esterase D also could not be detected within the tumor by electrophoretic analysis (*12*), whereas the electrophoretic pattern of the red blood cells was interpreted as 1-0. The electrophoretic pattern was interpreted as 1-0 rather than 1-1 because of the reduced activity seen by electrophoresis and by quantitative assay of the enzyme.

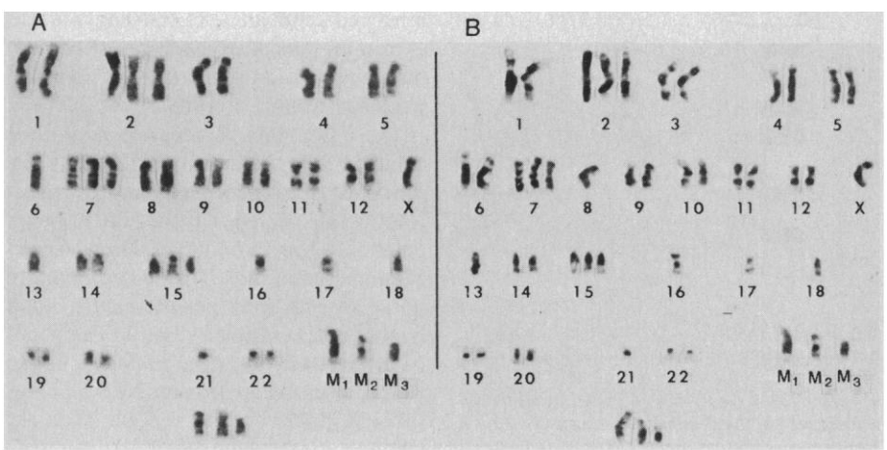

Fig. 2. Karyotypes from direct metaphase preparation of retinoblastoma cells from (A) the original tumor or (B) after two intraocular passages in the nude mouse, illustrating one of the two stem lines present in the original tumor. The cells had received 0.06 μg/ml of Colcemid treatment for 2 hours prior to their preparation and were subsequently trypsin-Giemsa–banded (*13*). Note the similarity in karyotypes and that one 13 chromosome is missing in each case.

Since her tumor cells contain only the deleted 13 chromosome they may provide valuable target cells for probes being developed to identify key genetic differences within 13q14 between normal 13 chromosomes and those which contain small deletions or mutations responsible for the susceptibility to retinoblastoma.

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Transfusions of Whole Blood Prevent Spontaneous Diabetes Mellitus in the BB/W Rat

Abstract. *Weekly transfusions of whole blood from a nondiabetic subline of BB/W rats reduced the incidence of diabetes in susceptible BB/W rats from 39 to 0 percent and the incidence of pancreatic insulitis from 64 to 6 percent. Responsiveness of lymphocytes to concanavalin A was found to be low in rats with diabetes or insulitis. Transfusion restored concanavalin A responsiveness to levels observed in control rats free of diabetes or insulitis. These data suggest that whole blood alters the course of autoimmune BB/W rat diabetes.*

Polyuria, polydipsia, polyphagia, and weight loss are the principal diagnostic features of human insulin-dependent diabetes mellitus (IDDM). Although the syndrome has been recognized for millennia, the etiology and prevention of diabetes remain enigmatic and elusive goals (*1*). The animal model that most closely mimics the human disease is the BB rat (*2*). As a model of IDDM it exhibits many features that are analogous to its human counterpart. The syndrome develops in 30 to 50 percent of diabetes-prone rats between 60 and 120 days of age. Diabetic rats are nonobese and develop acute ketoacidosis which is lethal within weeks unless treated with insulin.

Several lines of evidence suggest an immune pathogenesis of BB rat diabetes. These include the lymphocytic infiltration of pancreatic islets (insulitis) and prevention of the disease by immunosuppressive agents and neonatal thymectomy (*3*). Transplantation of neonatal bone marrow from another strain of rat also prevents diabetes in susceptible BB rats (*4*). In this last study, mixed lymphocyte cultures from the recipient BB rats demonstrated that they had been "immunologically reconstituted." This suggests that some form of immunodeficiency may be present in susceptible BB rats. The finding of lymphopenia in diabetic and insulitis-bearing BB rats supports this interpretation (*5*). Specifically, helper T lymphocytes alone (*6*), or both helper and suppressor T lymphocytes, may be present in abnormally low numbers in diabetic BB rats (*7*).

To test the hypothesis that deficiency of some circulating blood constituent is permissive to the development of diabetes, we administered whole blood transfusions to diabetes-prone rats. Donor blood from a nondiabetic subline of rats completely prevented the development of the disease in susceptible animals.

Five litters of BB/W diabetes-susceptible animals between 31 and 41 days of age were used (*8*). Rats from each litter were randomly assigned to one of two groups. Group 1 ($N=18$) received weekly transfusions of 1 ml of rat whole blood until the animals were 120 days of age (*9*). Group 2 ($N=18$) received 1 ml of normal saline injected on an identical schedule. Transfused blood was obtained from a line of BB/W rats in which neither diabetes nor insulitis has been observed in over seven generations (*10*). The animals were tested for glycosuria twice weekly from 60 to 140 days of age (*11*). Diabetes was diagnosed on the basis of 2+ glycosuria and plasma glucose in excess of 250 mg/dl (*12*).

When the rats were about 140 days of age, 2 ml of blood was withdrawn into heparinized capillary tubes from the orbital sinus of the transfused and nontransfused rats. The rats were then killed and the pancreata removed and fixed in Bouin's solution. Sections stained with hematoxylin and eosin were examined for the presence of insulitis by a pathologist (A.A.L.) who was not aware of the clinical or experimental status of the animals. The heparinized blood samples were processed to isolate lymphocytes which were then assayed for mitogenic responsiveness to concanavalin A (con A) according to previously described techniques (*13*). Blood from six additional 140-day-old rats from the nondiabetic subline was also used in the con A study.

Table 1 gives the frequency of diabetes in experimental and control animals (*14*). None of the animals that received transfusions became diabetic, whereas 39 percent of the nontransfused animals developed the syndrome. Table 1 also gives the frequency of insulitis among animals that did not become diabetic. Only one of 18 animals (6 percent) that had received the multiple blood transfusions demonstrated a mild focal insulitis. In the nontransfused group, 7 of 11 nondiabetic rats (64 percent) had insulitis.

Figure 1 illustrates the peripheral blood lymphocyte response to con A (*15*). The nontransfused animals with diabetes or insulitis are much less responsive than are nontransfused rats without insulitis or diabetes. The responsiveness of the latter group is comparable to that of rats from the nondiabetic subline. This