

Review Article

Retinoblastoma and the Genetic Theory of Cancer: An Old Paradigm Trying to Survive to the Evidence

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Retinoblastoma (Rb) is considered to represent the prototype of cancer linked to the sequential loss or inactivation of both alleles of a so-called “tumor suppressor gene”, the Rb1 gene. The pathogenetic mechanism behind this tumor was first hypothesized by Knudson in 1971 and further confirmed by others who identified the Rb1 gene whose loss or inactivation was claimed to be responsible for the disease. However, after about four decades of continuous research in the field of molecular biology, the evidence behind the role of the Rb1 gene in Rb appears to be seriously flawed in the light of epidemiological, biological, and clinical evidences. This editorial summarizes the inconsistencies on this subject. Nevertheless, the molecular biology establishment still adheres to the biased view of the genetic origin of Rb and other cancers, and hardly any alternative explanations are taken into account.

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1. Introduction

Retinoblastoma (Rb) is the most common intraocular malignant tumor in childhood, with an incidence of 1 in 15000 live births [1]. It may affect one eye (unilateral Rb) or both (bilateral Rb) during the first five years of life. Rare cases have also been reported in young adults [2, 3]. Although extensive epidemiologic studies have been done to study this tumor, the results have been more often misinterpreted at the expense of mutation theory which has prevailed until recently in spite of the outstanding evidence against it [4]. In the present review the authors analyze the most relevant epidemiological issues concerning retinoblastoma, in the light of recent developments highlighting the role of aneuploidy and genetic instability [5] in the pathogenesis of this eye cancer.

2. Historical Background

The most important studies to investigate the pathogenesis of retinoblastoma began with a paper published by Knudson in 1971 [6], when the author, after investigating the age distribution and laterality of a cohort of 48 Rb patients, concluded

that the disease could be inherited and formulated the so-called “two-hit theory” in order to explain its pathogenesis. In reality, no clues about the inheritance of retinoblastoma could be deducted from such a small sample. In fact, in his first report on this matter, Knudson referred to earlier, smaller series showing that, in retinoblastoma survivors with bilateral disease, the proportion of affected offspring closely approximated 50%, as in dominant (Mendelian) inheritance [6]. From an original, mathematical analysis of the above data, Knudson inferred that retinoblastoma is caused by two sequential (two hit) mutational events. According to this hypothesis, in the dominantly inherited form of the disease, one mutation is inherited via the germinal cells and the second spontaneously occurs in somatic cells of the retina and other tissues of the body. On the contrary, in the nonhereditary form, both mutations occur in the somatic (retinal) cells. The different timing and cell type involved by the two mutations determines the different clinical phenotype, with all bilateral and a minority of the unilateral cases being classified as hereditary, and the remaining unilateral cases being included in the sporadic group (Table 1). During the following forty years of epidemiological, clinical, genetic, and biological research in this field, with the discovery

TABLE 1: Distribution of retinoblastoma by type and laterality [6].

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

of the Rb1 as the prototype tumor suppressor gene, the medical establishment agreed on the pathogenetic “two-hit” theory which was further expanded by Knudson, in many other scientific articles and review papers [6–24]. This generated the widespread conviction that Rb is caused by two mutational events leading to the loss or inactivation of both alleles of the Rb1 gene, as still believed by some authors [25].

3. The Weak Foundations of the “Two-Hit” Theory

As mentioned above, the original input into the possible genetic derivation of retinoblastoma was based on limited evidence showing an apparently dominant Mendelian distribution of the disease in the offspring of bilaterally affected individuals, thus allowing Knudson to conclude that bilateral Rb is inherited through the germ cells. Minimum or no disagreement had been appeared on this account in the literature during the last four decades.

In an attempt to elucidate this issue, we have performed an analysis of the distribution of the disease in the offspring of unilaterally affected Rb survivors, referred to the Department of Ophthalmology—Ocular Oncology Unit at the University of Siena in Siena, Italy. We discovered that in a total of 16 children born to 12 unilaterally affected patients, 8 (50%) were healthy and 8 (50%) affected with Rb (Table 2). Using the reasoning of Knudson, it would be easily concluded that the unilateral disease phenotype is inherited and not sporadic, and this would be in sharp contrast with the current knowledge according to which bilateral Rb is “always” hereditary and unilateral Rb is “almost always” sporadic.

4. Familial Rb with Unilateral Phenotype: Is There Any Explanation?

It was reported by Knudson [6] and confirmed by others [26] that about 10% of all Rb cases do have a “positive family history”. In other words, the family history of the “index” case offers at least one other affected member, either a parent or another close relative. In this case, it is assumed that the events leading to the inactivation of the Rb1 gene run in the family, and therefore the first “hit” is transmitted through the germline, exactly what happens in bilateral Rb, but with one difference; bilateral Rbs, which after Knudson are all to be considered “hereditary”, are also assumed to have inherited the “first hit” through a mutation in one of the parent’s germ cells, but they are the only affected members in their families. We should therefore be reasoning that, since familial Rbs share the same pathogenetic mechanism with

TABLE 2: A list of 36 retinoblastoma survivors and their offspring referred to the Ocular Oncology Unit of the Department of Ophthalmology of the University of Siena (Italy). Unilaterally affected survivors generated a total of 16 children, half of which resulted affected.

Patient's number	Lat.	Offspring	
		Number of healthy	Number of affected
1	B		1
2	B	2	1
3	B	1	2
4	U	1	2
5	B		2
6	B	2	1
7	B		1
8	B	2	1
9	B	1	1
10	B		1
11	B		2
12	U		1
13	B		1
14	B		1
15	B		1
16	B		1
17	U		1
18	B		1
19	B		1
20	B		1
21	U		1
22	U		1
23	U		1
24	B		1
25	U		1
26	U	1	
27	B	1	
28	B	1	
29	B	1	
30	U	3	
31	B	2	
32	B	1	
33	B	1	
34	U	1	
35	U	1	
36	U	1	

bilateral (hereditary) Rb, the vast majority of these cases should show the bilateral phenotype. To be more accurate, we could make a calculation of the percentage of familial Rbs carrying the unilateral phenotype. As a matter of fact, Table 1 shows that the unilateral phenotype accounts for about 1/3 of all hereditary cases (or about 30%), and since familial Rb represents the 10% of all Rbs, it comes out that Rbs carrying the unilateral phenotype, within the familial group, should not be more than 3% (i.e., the 30% of the 10%). As a matter of fact, a meta-analysis of a cohort of 3584 patients

TABLE 3: A meta-analysis of literature shows that within a total of 344 familial retinoblastoma, 83 (23%) showed the unilateral phenotype.

Author	uRB	bRB	Tot.	Fam. U	Fam. B	Tot. Fam.
Abramson	626	905	1531	36	150	186
Gunalp	441	195	636	10	24	34
Sanders	282	149	431	15	38	53
Matzunaga	403	196	599	11	17	28
Hadjistilianou	227	160	387	11	32	43
Tot.	1979	1605	3584	83	261	344
				(23%)	(77%)	

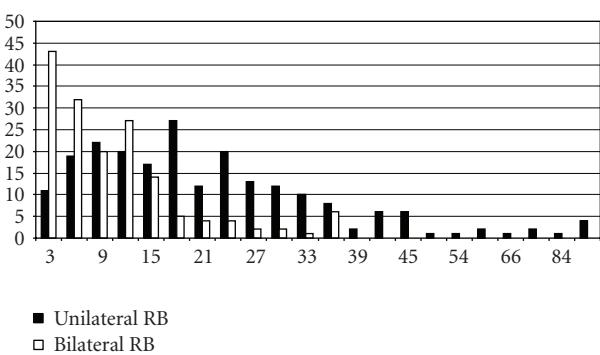


FIGURE 1: The distribution of retinoblastoma by laterality in a sample of 387 patients referred to the Ocular Oncology Unit of the Department of Ophthalmology of the University of Siena (Italy). Five cases, diagnosed beyond the age of 87 months, are not reported in the diagram. As it can be appreciated, the distribution of unilateral Rb by age at diagnosis is highly skewed, and therefore, the calculation of the “mean” in this sample may lead to unreliable inferences.

(Table 3), reported by us elsewhere [4, 5], reveals that on a total of 344 (9.5%) familial cases, 83 (24%) show the unilateral phenotype, instead of the predicted 3%, a rather unexplainable figure, in the light of the predictions made by the “two hit” hypothesis.

5. 13q Deletion Syndrome: A Case against and Not in Favour of the “Two-Hit” Hypothesis

In 1986, Potluri and coworkers observed that the association of Rb with the constitutional chromosome 13q deletion syndrome and the finding of 13q deletions or monosomy 13 in Rb cells in individuals with normal constitutional karyotypes seemed to suggest that chromosome 13q could contain a gene responsible for tumor development in retinoblastoma [27]. Although the authors themselves acknowledged that other chromosome abnormalities, in addition to those involving chromosome 13, are evident in retinoblastoma (additional copies of 1q material in 44% of cases, isochromosome 6p, in 45% of cases, monosomy 16, in 18% of cases, marker 1p+, in 13% of cases, and homogeneously staining regions and double minutes, in 9% of cases), further investigations on this matter stressed the role of 13q deletions in the genesis of Rb

TABLE 4: 13 cases of 13q deletion syndrome referred to the Ocular Oncology Unit of the Department of Ophthalmology of the University of Siena (Italy). 9/13 expressed the unilateral disease phenotype. The calculation of the mean age at diagnosis reveals a value of 10 months.

Patient	Gender	Age at diagnosis	Laterality
Z. A.	F	9 m	B
B. S.	M	5 m	B
V. F.	F	16 m	U
D. D. C.	F	10 m	B
S. G.	F	29 m	U
M.C.	M	10 m	U
F. I.	F	5 m	U
Z. S.	M	8 m	U
P. R.	F	8 m	U
P. A	M	12 m	U
Z. M.	F	5 m	U
Z. E.	F	9 m	U
L. I.	F	5 m	B

[28], thus reinforcing the belief that the loss or inactivation of the Rb1 was the only responsible for Rb to develop. But it is well known that retinoblastoma is only one among many different tumors associated with deletions of chromosome 13. Cancers linked with these deletions include chronic lymphocytic leukaemia (CLL) [29], chronic myeloproliferative disorders [30], multiple myeloma [31], hepatocellular carcinoma (HCC) [32], nasopharyngeal carcinoma [33], benign and low-grade malignant lipomatous tumors [34], bladder cancer [35], malignant mesothelioma [36], and prostate cancer [37]. The same pleiomorphism in the phenotypic expression of cancers associated with Rb1 gene mutations [38] is therefore evident in 13q deletion syndrome.

But the most important consideration to be made about the association of 13q deletion syndrome and Rb concerns the evident discrepancy existing between the expected and the real number of bilateral tumors among the patients affected by this genetic disorder. As a matter of fact, the 13q deletion syndrome must be confirmed by the cytogenetic analysis of peripheral blood lymphocytes, and it is due to a “constitutional” deletion of the long arm of chromosome 13 which involves, by definition, the Rb1 gene locus. Since the “constitutional” deletion of the Rb1 gene can only be present if transmitted through the germ cells of one parent, it follows that all patients affected by 13q deletion syndrome and retinoblastoma belong to the “hereditary” group of Knudson’s and must, therefore, express the bilateral disease phenotype. It happens, however, that this assumption does not fit the clinical reality. In Table 4 a series of 13 cases of 13q deletion syndrome and retinoblastoma referred to us over the last four decades is reported. Of these patients, only 4 had the bilateral disease phenotype, while the remaining 9 were unilaterally affected (Table 4). The mean age at diagnosis in this group, which is about 10 months, further reinforces the assumption that they must belong to the “hereditary” group of Knudson’s, but the unilateral disease phenotype is

unexplainably high. In the light of the “two hit” theory, the data summarized above do not have any rationalization, and the only plausible conclusion is that the assumptions made in regard to the role of the Rb1 gene in retinoblastoma are incorrect.

6. Concluding Remarks

Clinical Epidemiology is a leading discipline in the understanding of disease pathogenesis and etiology, but, as any other scientific endeavor, it relies on the correct interpretation of the available data. The proper analysis of data, in turn, relies not only on the individual researcher’s skill but also on social, economic, and political environment in which the data are analyzed. The presumed genetic origin of Rb and its relationship with the Rb1 gene represent a clear example of how an entire body of prominent researchers may fail to question a flawed pathogenetic hypothesis (i.e., the “two-hit” theory), for the sake of personal, academic, or other interests. It was not by chance that we had to approach many different scientific journals to have access to the medical community about the role of aneuploidy and genomic instability in the genesis of Rb [4, 5, 39]. We are still optimistic, however, because our alternative pathogenetic explanation has finally appeared in recent ophthalmologic literature [40].

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