The Epigenetic Origin of Retinoblastoma

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Abstract

The aim of the present review is to give new insights into the pathogenesis of retinoblastoma, by applying the principles of epigenetics to the study of clinical, epidemiological and biological data concerning the disease. As an emerging new scientific approach linking the genome to the environment, epigenetics, can not only explain the inconsistencies of the mutational ('two hit') model in the genesis of retinoblastoma, but also open new outstanding scenarios in the fields of diagnosis, treatment and prevention of this eye tumour and cancer in general. After more than four decades of predominance of the 'genetic' theory, this review represents, to the authors' knowledge, the first attempt to look at retinoblastoma from the point of view of epigenetics. The epigenetic model in the genesis of retinoblastoma, proposed herein, emphasises the role of environment and its interactions with the genome, in generating retinoblastoma in young children. Environmental toxicants, including, among others, radiations, wrong diets and infectious diseases, all play a major role in conditioning the degree of DNA methylation (one of the leading mechanisms of epigenetic gene regulation) in embryos and foetuses during pregnancy, thus leading to stable, functional alterations of the genome, which can be transmitted from one generation to the next, thus mimicking a hereditary disease. An accurate analysis of the currently available literature on both retinoblastoma and epigenetics, coupled with the knowledge of the variegated phenotypic expression of the disease, can easily lead to the conclusion that retinoblastoma is an epigenetic, rather than a genetic disease.

Keywords

Retinoblastoma, epigenetics, DNA methylation, histone acetylation/deacetylation

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About 'Causation' in Retinoblastoma

Since the formulation of the 'two hit' hypothesis in the genesis of retinoblastoma (Rb),[1] there has been widespread agreement, among researchers in the field of ocular oncology, that this eye tumour is determined by the loss, mutation or inactivation of both copies of one and a single gene. The gene was later identified and denominated Rb1,[2] and this discovery opened the 'hunting' to the inactivating mutation/s 'causing' Rb.

The 'causative' role of the *Rb1* gene mutations in Rb, is no longer a matter of discussion, among the vast majority of researchers worldwide, as it is clearly stated in both the most recent scientific papers[3–5] and institutional information[6–10] concerning the disease. However, it is widely recognised that the 'cause' of most (if not all) cancers is still unknown and the 'mutation theory' only tells us about a possible pathogenetic mechanism (the alteration of the DNA structure of the genome) presumably involved in the genesis of Rb. Nevertheless, the question about what ultimately 'causes' the mutations, which lead to cancer development in Rb (and in cancer, in general), remains unanswered.

Moreover, the role of gene mutations in the genesis of cancer, has been largely questioned by scientists who have remarked, among others, the fact that carcinogens do exist, as pesticides,[11] phenobarbital and clofibrate,[12] tumour promoters (1,4-dichlorobenzene), endocrine-modifiers (17β-estradiol), receptor-mediators (2,3,7,8-tetrachlorodibenzo-p-dioxin), immune suppressants (cyclosporine) or inducers of tissue-specific toxicity and inflammatory responses (metals such as arsenic and beryllium), among others, which are not genotoxic and therefore must be assumed to induce cancer without producing gene mutations.[13–17] These non-genotoxic (or 'epigenetic') carcinogens are well-known chemicals which induce cancer without modifying the DNA structure and nucleotide sequence of the human genome,[18] and represent the strongest argument against the role of gene mutation as the only pathogenetic mechanism leading to cancer.

However, the arguments against the role of gene mutations in cancer abound in the literature[19–27] even though a detailed discussion of this matter goes beyond the scope of the present review.

In summary, the awareness that gene mutations are only part of the process through which normal cells become cancerous, the other being represented by epigenetic (and 'non-structural') gene modifications, should suggest more caution in attributing a causative role to a mutation affecting one and a single gene, as it has been the case of Rb, after the first formulation of the two hit hypothesis, proposed by Knudson in 1971.

Epigenetics – The New Frontier in Cancer

The term 'Epigenetics' was coined in 1940 by Conrad Waddington to designate, "… the interactions of genes with their environment which bring the phenotype into being",[28,29] thus clearly indicating that

the phenotype (i.e. the external appearance) of a cell depends on the interplay of its genes with the environment surrounding it.

From the point of view of cancer research, epigenetics reminds us that when looking for the causes of cancer, one should never forget the fundamental role of the environment[30] since, "Overwhelming evidence indicates that the predominant contributor to many types of cancer is the environment".[31]

Literally, 'epi' means 'above' and therefore the term epigenetics (i.e. above genetics) designates events which modify gene expression without modifying the structure of the genes themselves.[29,32] Once again, from the standpoint of cancer research, this peculiar aspect of epigenetics reminds us that gene mutations are only one of the possible pathogenetic mechanisms through which the environment transforms normal cell into cancerous ones, the other being represented by non-structural modifications of gene expression.[33–35] These modifications are realised through different mechanism, including, among others, DNA methylation, covalent histone modifications, nucleosome positioning and histone variants, and microRNA, all of which have been already investigated in great detail.[36–41]

For the purposes of the present discussion, it will be important to mention that through hypermethylation of certain portions of the DNA, the function of entire genes (including tumour suppressor genes) can be suppressed,[39] and through hypomethylation, imprinted and/or 'silenced', as well as growth promoting genes can be activated.[42] In other words, the DNA methylation 'landscape' of cancer is profoundly distorted if compared to that of the normal cell, even if the DNA structure itself is intact.[38]

Furthermore, the work in the field of epigenetics, has shown that the inheritance of DNA sequences is not the only mechanism underlying the transgenerational transmission of physical, behavioural, and emotional traits in mammals.[43] An epigenetically modified DNA methylation landscape, is stable and can be transmitted from one generation to the next.

More importantly, the 'epigenome' (i.e. a genome-wide map of epigenetic modifications)[44] can be modulated by a variety of environmental factors, including chemicals, nutrition, and early environment, as well as by ageing. The epigenome, therefore, provides an important interface between genes and the environment and may be viewed as a potential mechanism for a rapid form of environmentally driven transgenerational adaptation.[45–51]

Finally, a growing body of evidence suggests that epigenetic gene regulation, including DNA methylation and histone modifications, are not only modulated by the environment, but may play a role in the foetal basis of adult disease.[29,32,52,53]

Epigenetis – Relevance to Retinoblastoma

Although cancer is commonly viewed as a disease linked to the environment,[31] Rb has, for a long time, represented an exception. As a matter of fact, since the formulation of the two hit hypothesis, cancer is generally viewed as a genetic, rather than an epigenetic disease. But, as we have seen in the previous paragraphs, the gene mutation in itself is neither necessary nor sufficient to explain how tumours develop and grow in human beings: it is not necessary, because epigenetics tells us that gene expression can be stably modified by the environment, in the total absence of structural changes (mutations) of the genomic DNA, and it is not sufficient because:

- A – cancer is a multistep process;[54]
- B – multiple and more complex phenomena (such as, for example, aneuploidy) must take place before cancerous transformation and progression take place;[20–27,54]
- C – the mutation in itself must have some underlying, though yet unknown, cause/s, and as such, cannot be considered causative, in cancer.

In a series of previously published papers,[55–59] the authors have argued against the mutation model in Rb, by highlighting the discordance between some of the predictions made by the two hit model and the available clinical data, but did not propose a clear cut alternative to the mutation theory.

Herein the authors propose a few arguments, which in the authors' opinion, demonstrate that epigenetics (and environment) play a crucial role in the genesis of Rb.

The Methylation Landscape of Retinoblastoma

Aberrant retinoblastoma 1 (pRb) pathway activity, resulting from defects in pRb (the protein produced by the *Rb1* gene), cyclin-dependent kinase inhibitor 2A (p16 INK4a), cyclin D1 (CCND1), or cyclin-dependent kinase 4 (CDK4), is observed in the majority of human sporadic cancers.[60–62] This pathway is commonly altered early in cancer development, indicating an ability to predispose cells to tumourigenesis.[63]

Given its ubiquitous expression in human tissues and its function of cell cycle control,[63,64] the *Rb1* gene fits the requisites to be included in the group of so called 'housekeeping' (HK) genes.[65,66]

As reported above, mutations are not the only mechanism through which the *Rb1* gene is inactivated in human cancer; namely, *in vitro* methylation of the promoter region of the *Rb1* gene, has been shown to dramatically reduce pRb expression[67] particularly in sporadic Rb which, on the other hand, is the most commonly known form of nonhereditary disease.

Moreover, methylation of the promoter regions of HK genes is a common mechanism that contributes to inactivating cell cycle control related genes (*Rb1*, among others) in the early stages of development of glial tumours.[69]

Interestingly, as a key gene in cell cycle control, *Rb1* has been found aberrantly methylated, alone or together with other cell cycle regulating genes, in different types of cancer, such as among others; glioblastoma[70] and other nervous system tumours,[71] epithelial odontogenic tumours,[72] bladder cancer,[73] radon-induced rat lung tumours,[74] follicular lymphoma,[75] SV40 associated B cell lymphoma,[76] gastric carcinoma,[77] neuroblastoma,[78] pituitary adenoma[79] and malignant fibrous histiocytoma.[80] Finally, Rb frequently shows aberrant methylation of other genes such as HIN-1,[81] HIC-1,[82] Caspase eight and 10[83] and RASSF1A,[84] all of which are commonly considered key genes in the development of cancer in young children. What does all this mean?

There is little doubt that DNA methylation, a key mechanism in epigenetic gene modulation, together with other mechanisms, such as histone acethylation, is involved in the genesis of cancer[85–90] and the finding of an altered methylation landscape of the *Rb1* and other important genes, in Rb, represents a clear indication of the role of epigenetics and environmental influences, on the genesis of this tumour.

Phenotypic 'Plasticity' and Retinoblastoma Epigenetics

Rb is not a single clinical entity, but rather a group of neoplastic affections of the retina showing various degrees of involvement of one or both eyes and also a sometimes extraordinarily different clinical behaviour.

As an example, 'retinoma' is considered a benign form of Rb or, in other words, a benign proliferation of an early retinal cell, caused, according to the mutational model, by the same mutation/s of the *Rb1* gene which is also responsible for the full blown disease.[91]

Although different hypotheses have been made to explain the 'unreliable' expression of the mutation affecting the *Rb1* gene (including, among others, the fuzzy concept of 'low penetrance'), advances in Rb genetic research[92] have demonstrated that even severe inherited mutations segregating in families with bilateral Rb patients, can cause retinoma.[93]

But, in the light of the genetic theory, the 'spontaneously regressed' Rb represents an even more challenging puzzle. The complete, spontaneous tumour necrosis leading to 'cure', is a well described phenomenon which is said to occur more frequently in neuroblastoma and Rb than in any other known malignant cancer.[94] The observation of spontaneous regression of retinoblastoma, dates back to 1956,[95] (i.e. more than a decade before the formulation of the mutational ('two hit') model). Further evidence has shown that 'spontaneously regressed retinoblastoma' is a rather common event[96–103] and actually malignant transformation of a spontaneously regressed tumour is also reported,[104] thus indicating the inherent plasticity of the cancer phenotype in Rb.

Other clinical phenotypes of the disease include: diffuse infiltrating Rb,[105,106] unilateral and bilateral Rb,[1,7,8,55–59] and also 'trilateral retinoblastoma',[107–109] in which a bilateral Rb is associated with intracranial tumours involving the pineal region.

How a mutation affecting one and a single gene (Rb1) could produce such a variety of clinical phenotypes, remains unexplained, on the basis of the mutational theory, unless the conceptual trick of 'penetrance' is used.

Penetrance is defined as "the proportion of individuals of a particular genotype that express its phenotypic effect in a given environment";[110] as remarked by Opitz[111] and Lenz, before him, "When the frequency of a trait in close relatives of probands does not agree very well with the assumption of [segregation] of a dominant gene, the discrepancy is addressed with the word 'penetrance'. However this word does not convey any special physiological or genetic explanation. It is nothing more than a verbal circumscription of the discrepancy between observation and assumption."

Although penetrance has always been matter of discussion, in Rb,[112] and various speculative hypotheses have been proposed for low penetrance Rb,[113] penetrance in itself is a rather undefined concept, which does not correspond to any known biochemical/molecular mechanisms and is presently viewed as a pure stochastic (but still unexplained) fluctuation in gene expression.[114]

A more appropriate term, to describe the variable expression of a given cancer phenotype, is that of 'phenotypic plasticity', usually defined as a property of individual genotypes to produce different phenotypes when exposed to different environmental conditions.[115]

In Rb, as we have seen, the same (presumed) mutation is responsible for sometimes extremely different clinical phenotypes and the reversibility of a pathological into normal phenotype is also a rather common event. This behaviour can be ascribed to phenotypic plasticity, which is not explained by the genetic theory, but is, instead, an integral part of the epigenetic theory of cancer.[116]

As a matter of fact, epigenetics, by linking the variable and potentially reversible effects of the environment to disease in general,[117,118] and cancer in particular,[119–122] represents the only possible explanation of phenotypic variation in health and disease and also offers important new tools to understanding how the interaction between the environment and the genome, leads to disease.[42]

Trans Generational Inheritance of Epigenetic (Acquired) Alterations and Retinoblastoma

There is cumulating evidence that chemical toxicants have detrimental effects not only on individuals directly exposed to the toxicant but also on their offspring.

Diethylstilbestrol, a synthetic non-steroidal oestrogen prescribed in the 1970s to prevent miscarriage in women with prior history, helped pregnancies to go to term, but also induced severe developmental abnormalities and increased the risk for breast cancer and a rare form of adenocarcinoma in girls whose mothers were exposed to the drug during the first trimester of pregnancy;[123] in other words, the risk of cancer appeared to be transmitted to the following generation.

Similar, transgenerational effects were obtained in mice, in which perinatal exposure to the drug, produced abnormalities in uterine development and uterine cancer in first and second generations. These abnormalities were suggested to result from aberrant DNA methylation of a gene controlling normal uterine development.[124]

Vinclozolin, a fungicide commonly used for agricultural fruit crops, increases the incidence rate of tumour formation in ageing males exposed to it prenatally (F1) and their offspring (F2–F4).[46]

But the transgenerational extension of the detrimental effects of the environment on the epigenome (basically the DNA methylation landscape), also seem to apply to diet (either low protein or high fat), and poor early environment (childhood trauma or abuse), thus showing that the environment can stably impress its effects on the genome of several generations in the offspring of the first individual exposed; changes in the epigenome, established by the environment during early development, may be reversed by environmental stimuli even in adults, emphasising the plasticity of DNA methylation (and cancer phenotypes) in the adult.[43]

Regarding Rb, it is known that, with the only the exception of the familial group (8–10 % of all cases), in which the disease is found in

the 'proband' and in some of his/her relatives, the hereditary (bilateral) form of the disease is, according to the two hit model, a sporadic Rb (since no other affected family member can be identified) determined by a germline mutation.

In terms of epigenetic gene regulation therefore, Rb is still to be viewed as an inherited disease, but the inheritance concerns, in this case, the transmission of acquired environmental negative effects on the genome, through several generations.

More importantly, transgenerational inheritance of environmental effects, is maintained and detectable in at least the F3 generation, where F0 is the gestating mother exposed, F1 is the embryo and F2 are the embryo's germ cells. In other words, when the gestating female (F0) is exposed to toxicants, both F1 (embryo) and F2 (embryo's germ cells) are also directly exposed. Therefore, disease phenotypes in the F1 and F2 generations might still be due to the direct exposure of F0, F1 and F2 to environmental toxicants.[29,32,52,53]

Regarding the transgenerational transmission of environmental effects on the genome, in Rb, it is extremely significant that clinical reports on the disease are almost invariably limited to retinoblastoma patients (F1) and rarely to their first generation descendants (F2), while a retinoblastoma occurring in the F3 generation, according to the mutational model proposed by Knudson, belongs to the familial group. All this implies that the (presumed) transgenerational effects of a mutation on the expression of cancer phenotype, in retinoblastoma, are indistinguishable from those produced on the epigenome by the environment.

In conclusion, Epigenetics tells us that the 'inheritance' of the cancer phenotype, does not necessarily depend on mutational events affecting one or more genes, but may rather be the result of more complex and still incompletely understood (functional) interactions between DNA and the environment.

Retinoblastoma, Inheritance and Environment – The Navajo Paradigm

The incidence of hereditary (bilateral) Rb is rather invariant throughout the world, but that of the sporadic form of the disease, shows wide variations, being more frequent in less industrialised countries and in less affluent populations, thus indicating that the appearance of a sporadic retinoblastoma is most probably conditioned by non-genetic factors such as poor living conditions and other environmental agents *in utero*.[125,126]

Environmental factors, such as diet[127] and infectious agents,[128] as well as ionising radiation[129] have been reported to play a role in the genesis of Rb. However, the mechanism through which this is achieved is not a direct structural DNA damage, but rather a stable modification of the epigenome, which is transmitted through the germ cells.[117–122]

Regarding the role of low level radiation, of extreme interest is the case of the American Indian Navajo population who has represented the main working force in the uranium mines of South-west America, from World War II until 1971[130] and still live in villages located near the mines. The incidence of Rb among these populations is more than twice when compared to other world populations.[131] More importantly, the incidence seem to arise to 20 times in the village of Seascale, situated in the vicinity of a nuclear reprocessing plant, and best known in epidemiological circles for its longstanding high incidence of malignant diseases in young people.[132] The paper published by Stiller, on this subject, is of great interest since, although radiation is known to induce DNA mutations, it clearly argues against the mutational genesis of Rb. In particular it refers to a group of five unilateral sporadic Rb, born to mothers who had lived in Seascale, a village situated near the Sellafield nuclear reprocessing plant, in the region of Cumbria. The puzzling feature of these five cases is that none of them have been diagnosed among children who were themselves resident in Seascale, although the observed incidence among children of mothers who had previously lived there has been calculated to be about 20 times expected. Interestingly, "… even if the risk is 20 times that in the rest of Britain", observes the author, "only one case of Rb would be expected in Seascale every 40 years".

The above data, further confirmed by other short reports[133–135] on the same subject, represent a rather typical circumstance for epigenetics which explains the ability of certain chemical compounds (or low levels radiation) to initiate biological perturbations that can lead to malignancy, despite being weak mutagens or lacking mutagenic activity altogether.[136–137]

Concluding Remarks and Future Perspectives

Traditionally, cancer has been viewed as a genetic disease, although recent advances in the field of epigenetics show that cancer is instead the manifestation of both genetic and epigenetic modifications.[138]

Classic genetics alone has been shown largely insufficient to explain the diversity of phenotype within a population and why, despite their identical DNA sequences, monozygotic twins or cloned animals show different phenotypes and disease susceptibilities.[139]

Epigenetics can be described as a stable alteration in gene expression potential that takes place during development and cell proliferation, without any change in gene sequence. This is most commonly (but not exclusively) achieved through DNA methylation, which is one of the most commonly occurring epigenetic events in the mammalian genome.[90]

Alterations of the gene methylation profile in cancer include either hypo- or hyper methylation, which in turn, encompass both global and/or gene specific modifications. Global hypomethylation is responsible of the increased chromosomal instability and tumour frequency with age, while gene-specific hypomethylation leads to oncogene activation. In addition, the silencing of tumour-suppressor genes is associated with promoter DNA hypermethylation and chromatin hypoacetylation, which affect diverse genes, including Rb1.[140] Although epigenetic regulation of gene expression is the mechanism through which the extraordinary variety of specialised cells of the body differentiate starting from a single undifferentiated ancestor,[141] the relevance of epigenetic factors in disease in humans was first detected only in 1983 when Feinberg and Vogelstein found that gene hypomethylation could distinguish some human cancers from their normal counterparts.[142]

Today, deregulation of gene expression is widely considered a hallmark of cancer[35,36] and although genetic lesions have been the focus of cancer research for many years, as in the case of Rb, it has

become increasingly recognised that aberrant epigenetic modifications play major roles in all cancers, including Rb.[143]

As the authors have shown herein, the mutational model is largely inadequate to explain both the variegated expression and inherent plasticity of the clinical phenotype in Rb, while epigenetics offers a more coherent and reliable paradigm. Also and more importantly, this paradigm shift (from genetic to epigenetic), in Rb, opens up other important avenues in cancer research which promise to revolutionise the fields of both ophthalmology and oncology; namely:

- The potential reversibility of epigenetic states offers exciting opportunities for novel cancer drugs that can restore epigenetically silenced cancer genes. DNA methyltransferases and histone deacetylases are the two major drug targets for epigenetic inhibition to date, although others are expected to be added in the near future.[144–146]
- Epigenetic changes in cancer cells not only provide novel targets for drug therapy but also offer unique prospects for cancer diagnostics, through the study of gene expression, the evaluation of histone modifications and chromatin protein composition, and also the analysis of the promoter DNA methylation status.[139,147–149]
- Finally and more importantly, by shifting the focus on the environment and the complex interactions between the environmental regulation of gene expression and the genome, rather than on the genes themselves, epigenetics stresses the importance of cancer prevention and the changes of most of our common lifestyles, including diet and behaviour. ■

1. Knudson AG, Mutation and cancer: statistical study of Rb, *Proc Natl Acad Sci U S A,* 1971; 68,820–3.
2. Friend SH, Bernards R, Rogelj S, et al., A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma, *Nature,* 1986;323:643–6.
3. Ahani A, Behnam B, Khorram Khorshid HR, et al., RB1 gene mutations in Iranian patients with retinoblastoma: report of four novel mutations, *Cancer Genet,* 2001;204:316–22.
4. Parsam VL, Ali MJ, Honavar SG, et al., Splicing aberrations caused by constitutional RB1 gene mutations in retinoblastoma, *J. Biosci,* 2001;36:281–7.
5. Chinnam M, Goodrich DW, RB1, development, and cancer, *Curr Top Dev Biol,* 2011;94: 129–69.
6. Vandezande KE, Gallie B, Rushlow D, Background for retinoblastoma families and those who provide care - Retinoblastoma Solutions. Molecular Diagnostic Laboratory, 2006. Certified by the College of American Pathologists. Available at: www.retinoblastomasolutions.org/info/FamilyLetter.pdf (accessed 13 April 2012).
7. Lohmann DR, Gallie BL, Retinoblastoma. In: Pagon RA, Bird TC, Dolan CR, et al. (eds), *Gene Reviews™* (Internet), University of Washington, Seattle, 2010. Available at: www.ncbi.nlm.nih.gov/books/NBK1452 (accessed 11 April 2012).
8. Online Mendelian Inheritance in Man®, #180200 RETINOBLASTOMA; RB1, 1986, Available at: http://omim.org/entry/180200 (accessed 11 April 2012).
9. American Cancer Society, Gene mutations that can lead to cancer, 2011. Available at: www.cancer.org/cancer/cancercauses/geneticsandcancer/oncogenesandtumorsuppressorgenes/oncogenes-tumor-suppressor-genes-and-cancer-mutations-and-cancer (accessed 11 April 2012).
10. National Cancer Institute, Slide 2: Cancer Genomics, 2005. Available at: www.cancer.gov/cancertopics/understandingcancer/cancergenomics/page2 (accessed 11 April 2012).
11. Rakitsky VN, Koblyakov VA, Turusov VS, Nongenotoxic (Epigenetic) carcinogens: pesticides as an example. A Critical Review, *Teratog Carcinog Mutagen,* 2000;20(4):229–40.
12. Diez-Fernandez C, Sanz N, Alvarez AM, et al., The effect of non-genotoxic carcinogens, phenobarbital and clofibrate, on the relationship between reactive oxygen species, antioxidant enzyme expression and apoptosis, *Carcinogenesis,* 1998;19(10),1715–22.
13. Hernández LG, van Steeg H, Luijten M, et al., Mechanisms of non-genotoxic carcinogens and importance of a weight of evidence approach, *Mutat Res,* 2009;682(2–3):94–109.
14. Van Hummelen P, Sasaki J, State-of-the-art genomics approaches in toxicology, *Mutat Res,* 2010;705(3):165–71.
15. Plant N, Can systems toxicology identify common biomarkers of non-genotopic carcinogenesis?, *Toxicology*, 2008;254(30)164–9.
16. Lima BS, Van der Laanc JW, Mechanisms of nongenotoxic carcinogenesis and assessment of the human hazard, *Regul Toxicol Pharmacol*, 2000;32(2):135–43.
17. Klanvnig SE, Klamendulis LM, Xu Y, Epigenetic mechanisms of chemical carcinogenesis, *Hum Exp Toxicol*, 2000;19(10):543–55.
18. Sivak A, Goyer MM, Ricci PF, Nongenotoxic carcinogens: prologue. In: Butterworth BE, Slaga TJ (ed), *Banbury Report 25: Nongenotoxic Mechanisms in carcinogenesis*, Cold Spring Harbor Laboratory, New York, 1987;1–8.
19. Jaffe LF, Epigenetic theories of cancer initiation, *Adv Cancer Res,* 2003;90:209–30.
20. Li RH, Sonik A, Stindl R, et al., Aneuploidy vs. gene mutation hypothesis of cancer: recent study claims mutation but is found to support aneuploidy, *Proc Natl Acad Sci U S A,* 2000;97:3236–41.
21. Duesberg P, Rausch C, Rasnick D, et al., Genetic instability of cancer cells is proportional to their degree of aneuploidy, *Proc Natl Acad Sci U S A,* 1998;95(23):13692–697.
22. Duesberg P, Li R, Rasnik D, et al., Aneuploidy precedes and segregates with chemical carcinogenesis, *Cancer Genet Cytogenet,* 2000;119:83–93.
23. Duesberg P, Stindl R, Li RH, et al., Aneuploidy versus gene mutation as cause of cancer, *Curr Sci,* 2001;81:490–500.
24. Duesberg P, Li R, Rasnick D, Aneuploidy approaching a perfect score in predicting and preventing cancer, *Cell Cycle,* 2004;3(6):823–8.
25. Duesberg P, Does Aneuploidy or mutation start cancer?, *Science,* 2005;307:5706.
26. Duesberg P, Li R, Fabarius A, et al., The chromosomal basis of cancer, *Cell Oncol*, 2005;27(5–6):293–318.
27. Duesberg P, Chromosomal chaos and cancer, *Sci Am,* 2007(5);296:52–9.
28. Waddington CH, Organisers and Genes, Cambridge University Press, Cambridge, 1940.
29. Dolinoy DC, Weidman JR, Jirtle RA, Epigenetic gene regulation: linking early developmental environment to adult disease, *Reprod Toxicol,* 2007;23(3):297–307.
30. Clapp RW, Jacobs MM, Loechler EL, Environmental and occupational causes of cancer: new evidence 2005-2007, *Rev Environ Health,* 2008;23(1):1–37.
31. An overview of the evidence on environmental and occupational determinants of cancer, Presented at: International Conference on Environmental and Occupational Determinants of Cancer: Interventions for Primary Prevention, WHO, Asturias (Avilés, Gijón), Spain, 17–18 March 2011. Available at: www.who.int/phe/news/events/international_conference/en/index.html (accessed 12 April 2012).
32. Dolinoy DC, *Epigenetic gene regulation: early environmental exposures Pharmacogenomics,* 2007;8(1):5–10.
33. Baylin SB, Ohm JE, Epigenetic gene silencing in cancer – a mechanism for early oncogenic pathway addiction?, *Nat Rev Cancer,* 2006;6(2):107–16.
34. Brait M, Sidransky D, Cancer epigenetics: above and beyond, *Toxicol Mech Methods*, 2011;21(4):275–88.
35. Rodríguez-Paredes M, Esteller M, Cancer epigenetics reaches mainstream oncology, *Nat Med,* 2011;17(3):330–9.
36. Sharma S, Kelly TK, Jones PA, Epigenetics in cancer, *Carcinogenesis,* 2010;31(1):27–36.
37. Ehrlich M, DNA hypomethylation in cancer cells, *Epigenomics,* 2009;1(2):239–59.
38. Lopez J, Percharde M, Coley HM, et al., The context and potential of epigenetics in oncology, *Br J Cancer,* 2009;100(4):571–7.
39. Taby R, Issa JP, Cancer Epigenetics, *CA Cancer J for Clin,* 2010;60(60):376–92.
40. Miremadi A, Oestergaard MZ, Pharoah PDP, et al., Cancer genetics of epigenetic genes, *Hum Mol Gen,* 2007;16:R28–R49.
41. Choudhuri S, Cui Y, Klaassen CD, Molecular Targets of epigenetic regulations and effectors of environmental influences, *Toxicol Appl Pharmacol,* 2010;245(3):378–93.
42. Feinberg AP, Phenotypic plasticity and the epigenetics of human diseases, *Nature,* 2007;447(7143):433–40.
43. Franklin TB, Mansuy IM, Epigenetic inheritance in mammals: evidence for the impact of adverse environmental effects, *Neurobiol Dis,* 2010;39(1):61–5.
44. Comment: Time for the epigenome, *Nature,* 2010;463(7281):587.
45. Anway MD, Cupp AS, Uzumcu M, et al., 2005. Epigenetic transgenerational actions of endocrine disruptors and male fertility, *Science,* 2005;308(5727):1466–9.
46. Anway MD, Leathers C, Skinner MK, Endocrine disruptor vinclozolin induced epigenetic transgenerational adult-onset disease, *Endocrinology,* 2006;147(12):5515–23.
47. Roth TL, Lubin FD, Funk AJ, et al., Lasting epigenetic influence of early-life adversity on the BDNF gene, *Biol Psychiatry,* 2009;65(9):760–9.
48. Waterland RA, Dolinoy DC, Lin JR, et al., Maternal methyl supplements increase offspring DNA methylation at Axin Fused, *Genesis,* 2006;44(9):401–6.
49. Weaver IC, Cervoni N, Champagne FA, et al., Epigenetic programming by maternal behavior, *Nat Neurosci,* 2004;7(8):847–54.
50. Weaver IC, Champagne FA, Brown SE, et al., Reversal of maternal programming of stress responses in adult offspring through methyl supplementation: altering epigenetic marking later in life, *J Neurosci,* 2005;25(47):11045–54.
51. Wilson VL, Jones PA, DNA methylation decreases in aging but not in immortal cells, *Science,* 1983;220(4601):1055–7.
52. Jirtle RL, Skinner MK, Environmental epigenomics and disease susceptibility, *Nat Rev Genet*, 2007; 8(4): 253–62.
53. Dolinoy DC, Das R, Weidman JR, et al., Metastable epialleles, imprinting, and the fetal origins of adult diseases, *Pediatr Res,* 2007;61(5 Pt 2):30R–37R.
54. Duesberg P, Li R, Multistep carcinogenesis: a chain reaction of aneuploidizations, *Cell Cycle* 2003;2(3):202–10.
55. Mastrangelo D, De Francesco S, Di Leonardo A, et al., Does the evidence matter in medicine? The Rb paradigm, *Int J Cancer,* 2007;121(11):2501–5.
56. Mastrangelo D, De Francesco S, Di Leonardo A, et al., Retinoblastoma epidemiology: does the evidence matter?, *Eur J Cancer,* 2007;43(10):1596–603.
57. Mastrangelo D, Hadjistilianou T, De Francesco S, et al., Retinoblastoma and the genetic theory of cancer: an old paradigm trying to survive to the evidence, *J Cancer Epidemiol,* 2009;2009,301973
58. Mastrangelo D, De Francesco S, Di Leonardo A, et al., The Retinoblastoma paradigm revisited, *Med Sci Monit,* 2008;14(12):RA231–40.
59. Mastrangelo D, Loré C, Grasso G, Retinoblastoma as an epigenetic disease: a proposal, *J Cancer Ther,* 2011;2(3):362–71.
60. Weinberg RA, The retinoblastoma protein and cell cycle control, *Cell*, 1995;81(3):323–30.
61. Sherr CJ, Cancer cell cycles, *Science,* 1996;274(5293):1672–77.
62. Ortega S, Malumbres M, Barbacid M, Cyclin D-dependent kinases, INK4 inhibitors and cancer, *Biochim Biophys Acta,* 2002;1602,73–87.
63. Simin K, Wu H, Lu L, et al., pRb Inactivation in Mammary Cells Reveals Common Mechanisms for Tumor Initiation and Progression in Divergent Epithelia, *PLoS Biology,* 2004;2:E22.
64. Genetics Home Reference, RBI. Available at: http://ghr.nlm.nih.gov/gene/RB1 (accessed 13 April 2012).
65. Chang CW, Cheng WC, Chen CR, et al., Identification of human housekeeping genes and tissue-selective genes by microarray meta-analysis, *PLoS ONE,* 2011;6(7):e22859.
66. Genetics Home Reference, Housekeeping gene. Available at: http://ghr.nlm.nih.gov/glossary=housekeepinggene (accessed 13 April 2012).
67. Ohtani-Fujita N, Fujita T, Aoike A, et al., CpG Methylation inactivates the promoter activity of the human retinoblastoma tumor-suppressor gene, *Oncogene,* 1993;8:1063–7.
68. Ohtani-Fujita N, Dryja TP, Rapaport JM, et al., Hypermethylation in the retinoblastoma gene is associated with unilateral, sporadic retinoblastoma, *Cancer Genet Cytogenet,* 1997;98:43–9.
69. Bello J, Gonzalez-Gomez P, Eva Alonso M, et al., Methylation analysis of cell cycle control genes RB1, p14ARF and p16INK4a in human gliomas, Cancer Ther, 2004;2:187–94.
70. Nakamura M, Yonekawa Y, Kleihues P, et al., Promoter hypermethylation of the RB1 gene in glioblastomas, *Lab Invest,* 2001;81:77–82.
71. Gonzalez-Gomez P, Bello MJ, Alonso ME, et al., CpG island methylation status and mutation analysis of the RB1 gene essential promoter region and protein-binding pocket domain in nervous system tumours, *Br J Cancer,* 2003;88:109–14.
72. Moreira PR, Guimarães MM, Gomes CC, et al., Methylation frequencies of cell-cycle associated genes in epithelial odontogenic tumours, *Arch Oral Biol*, 2009;54:893–97.
73. Malekzadeh K, Sobti RC, Nikbakht M, et al., Methylation patterns of Rb1 and Casp-8 promoters and their impact on their expression in bladder cancer, *Cancer Invest,* 2009;27:70–80.
74. Bastide K, Guilly MN, Bernaudin JF, et al., Molecular analysis of the Ink4a/Rb1-Arf/Tp53 pathways in radon-induced rat lung tumors, *Lung Cancer,* 2009;63:348–53.
75. Chim CS, Wong KY, Loong F, et al., Frequent epigenetic inactivation of Rb1 in addition to p15 and p16 in mantle cell

and follicular lymphoma, *Hum Pathol,* 2007;38:1849–57.
76. Amara K, Trimeche M, Ziadi S, et al., Presence of Simian Virus 40 DNA sequences in diffuse large B-cell lymphomas in Tunisia correlates with aberrant promoter hypermethylation of multiple Tumor Suppressor Genes, *Int J Cancer,* 2007;121,2693–702.
77. Zhao YF, Zhang YG, Tian XX, et al., Aberrant methylation of multiple genes in gastric carcinomas, *Int J Surg Pathol,* 2007;15:242–51.
78. Michalowski MB, de Fraipont F, Plantaz D, et al., Methylation of Tumor-Suppressor Genes in Neuroblastoma: The RASSF1A gene is almost always methylated in primary tumors, *Pediatr Blood Cancer,* 2008;50:29–32.
79. Yoshino A, Katayama Y, Ogino A, et al., Promoter hypermethylation profile of cell cycle regulator genes in pituitary adenomas, *J Neurooncol,* 2007;83:153–62.
80. Binck U, T. Schlott T, S. Storber S, et al., Alterations of the Retinoblastoma and P16 pathway correlate with promoter methylation in malignant fibrous histiocytomas, *Anticancer Res,* 2006;26:3461–6.
81. Shigematsu H, Suzuki M, Takahashi T, et al., Aberrant methylation of HIN-1 (high in normal-1) is a frequent event in many human malignancies, *Int J Cancer,* 2005;113:600–4.
82. Rathi A, Virmani AK, Harada K, et al., Aberrant methylation of the HIC1 promoter is a frequent event in specific pediatric neoplasms, *Clin Cancer Res,* 2003;9:3674–8.
83. Harada H, Toyooka S, Shivapurkar N, Deregulation of Caspase 8 and 10 expression in pediatric tumors and cell lines, *Cancer Res,* 2002;62:5897–901.
84. Harada K, Toyooka S, Maitra A, et al., Aberrant promoter methylation and silencing of the RASSF1A gene in pediatric tumors and cell lines, *Oncogene,* 2002;21:4345–9.
85. Watanabe Y, Maekawa M, Methylation of DNA in cancer, *Adv Clin Chem,* 2010;52:145–67.
86. Shames DS, Minna JD, Gazdar AF, DNA methylation in health, disease, and cancer, *Curr Mol Med,* 2007;7:85–102.
87. Paz MF, Fraga MF, Avila S, et al., A systematic profile of DNA methylation in human cancer cell lines, *Cancer Res,* 2003;63:1114–21.
88. McGarvey KM, Greene E, Fahrner JA, et al., DNA methylation and complete transcriptional silencing of cancer genes persist after depletion of EZH2, *Cancer Res,* 2007;67:5097–102.
89. Ordway JM, Bedell JA, Citek RW, et al., Comprehensive DNA methylation profiling in a human cancer genome identifies novel epigenetic targets, Carcinogenesis, 2006;27:2409–23.
90. Das MP, Singal R, DNA Methylation and cancer, *J Clin Oncol,* 2004;22:4632–42.
91. Gallie BL, Ellsworth RM, Abramson DH, et al., Retinoma: spontaneous regression of retinoblastoma or benign manifestation of the mutation?, *Br J Cancer,* 1982;45:513–21.
92. Nichols KE, Walther S, Chao E, et al., Recent advances in retinoblastoma genetic research, *Curr Opin Ophthalmol,* 2009;20:351–5.
93. Abouzeid H, Schorderet DF, Balmer A, et al., Germline mutations in retinoma patients: relevance to low penetrance and low-expressivity molecular basis, *Mol Vis,* 2009;15:771–7.
94. Papac J, Spontaneous regression of cancer, *Canc Treat Rev*, 1996;22:395–423.
95. Steward JK, Smith JL, Arnold EL, Spontaneous regression of retinoblastoma, *Br J Ophthalmol,* 1956;40:449–61.
96. Sanborn GE, Augsburger JJ, Shields JA, Spontaneous regression of bilateral retinoblastoma, *Br J Ophthalmol,* 1982;66:685–90.
97. Lam A, Shields CL, Manquez ME, et al., Progressive resorption of a presumed spontaneously regressed retinoblastoma over 20 years, *Retina,* 2005;25:230–1.
98. Sinha N, Shields CL, Ramasubramanian A, et al., Giant spontaneously regressed retinoblastoma, *Can J Ophthalmiol,* 2010;45:89–90.
99. Dhir SP, Jain IS, Das SK, Regressed retinoblastoma, *Indian J Ophthalmol,* 1976;24:35–36.
100. Roberts BN, Pilz DT, Walters RF, Bilateral spontaneously regressed retinoblastoma with preservation of vision, *Eye,* 1997;11:122–4.
101. Benson WE, Cameron JD, Furgiuele FP, et al., Presumed spontaneously regressed retinoblastoma, *Ann Ophthalmol,* 1978;10:897–9.
102. Shirai K, Okada Y, Saika S, Immunohistochemical observation of anterior subcapsular cataract in eye with spontaneously regressed retinoblastoma, *J Cataract Refract Surg,* 2010;36:503–7.
103. Khodadoust AA, Roozitalab HM, Smith RE, et al., Spontaneous regression of retinoblastoma, *Surv Ophthalmol,* 1977:21:467–78.
104. Eagle RC, Shields JA, Donoso L, et al., Malignant transformation of spontaneously regressed retinoblastoma, retinoma/retinocytoma variant, *Ophthalmology*, 1989;96:1389–95.
105. Morgan G, Diffuse infiltrating retinoblastoma, *Br J Ophthalmol,* 1971;55:600–6.
106. Shields CL, Ghassemi F, Tuncer S, et al., Clinical spectrum of diffuse infiltrating retinoblastoma in 34 consecutive eyes, *Ophthalmology,* 2008;115:2253–8.
107. Cho EY, Suh YL, Shin HJ, Trilateral retinoblastoma: a case report, *J Korean Med Sci,* 2002;17:137–40.
108. Provenzale JM, Gururangan S, Klintworth G, Trilateral retinoblastoma: clinical and radiologic progression, *Am J Roentgenol,* 2004;183:505–11.
109. Kivela T, Trilateral retinoblastoma: a meta analysis of hereditary retino blastoma associated with primary ectopic intracranial retinoblastoma, *J Clin Oncol,* 1999;17:1829–37.
110. Merriam-webster dictionary, http://www.merriam-webster.com/dictionary/penetrance
111. Opitz JM, Some comments on penetrance and related subjects, *Am J Med Genet,* 1981;8:265–74.
112. Matsunaga E, On estimating penetrance of the retinoblastoma gene, *Hum Genet,* 1980;56:127–8.
113. Hung CC, Lin SY, Lee CN, et al., Low penetrance of retinoblastoma for p.V654L mutation of the RB1 gene, *BMC Med Genet,* 2011;76:2–5.
114. Raj A, Rifkin SA, Andersen E, et al., Variability in gene expression underlies incomplete penetrance, *Nature,* 2010;463:913–8.
115. Pigliucci M, Murren CJ, Schlichtin CD, Phenotypic plasticity and evolution by genetic assimilation, *J Exp Biol,* 2006;209:2362–7.
116. Richards CL, Bossdorf O, Pigliucci M, What role does heritable epigenetic variation play in phenotypic evolution?, *BioScience,* 2010;60:232–7.
117. Morison IM, Reik W, Nutrition, environment, and epigenetics. In: Symonds ME (ed), *Maternal – fetal nutrition during pregnancy and lactation*, Cambridge, UK: Cambridge University Press, 2010, 180–95.
118. Jaenisch R, Bird A, Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals, *Nat Genet,* 2003;33(suppl):245–54.
119. Herceg Z, Epigenetics and cancer: towards an evaluation of the impact of environmental and dietary factors, *Mutagenesis,* 2007;22:91–103.
120. Skinner MK, Environmental epigenetic transgenerational inheritance and somatic epigenetic mitotic stability, *Epigenetics,* 2011;6:838–42.
121. Nyström M, Mutanen M, Diet and epigenetics in colon cancer, *World J Gastroenterol,* 2009;15(3):257–63.
122. Weidman JR, Dolinoy DC, Murphy SK, et al., Cancer susceptibility: epigenetic manifestation of environmental exposures, *Cancer J,* 2007;13:9–16.
123. Palmer JR, Wise LA, Hatch EE, et al., Prenatal diethylstilbestrol exposure and risk of breast cancer, *Cancer Epidemiol Biomarkers Prev* 2006;15:1509–14.
124. Bromer JG, Wu J, Zhou Y, et al., Hypermethylation of Homeobox A10 by in utero Diethylstilbestrol exposure: an epigenetic mechanism for altered developmental programming, *Endocrinology,* 2009;150:3376–82.
125. Stiller C, Parkin D, Geographic and ethnic variations in the incidence of childhood cancer, *Br Med Bull,*1996;52:682–703.
126. Stiller CA, Epidemiology and genetics of childhood cancer, *Oncogene,* 2004;23: 6429–44.
127. Orjuela MA, Titievsky L, Liu X, et al., Fruit and vegetable intake during pregnancy and risk for development of sporadic retinoblastoma, *Cancer Epidemiol Biomarkers Prev,* 2005;14:1433–40.
128. Orjuela M, Castaneda VP, Ridaura C, et al., Presence of human papilloma virus in tumor tissue from children with retinoblastoma: an alternative mechanism for tumor development, *Clin Cancer Res,* 2000;6:4010–6.
129. Bunin GR, Felice MA, Davidson W, et al., Medical radiation exposure and risk of retinoblastoma resulting from new germline RB1 mutation, *Int J Cancer,* 2011;128:2393–404.
130. Brugge D, Goble R, The history of uranium mining and the navajo people, *Am J Pub Health,* 2002;92:1410–19.
131. Berkow RL, Fleshman JK, Retinoblastoma in Navajo indian children, *Am J Dis Child,* 1983;137:137–8.
132. Stiller CA, Retinoblastoma and low level of radiation, *Br Med J,* 1993;307:461–2.
133. Butler R, Flowerdew R, Gatrell AC, Retinoblastoma in children of former residents in Seascale, *Br Med J,* 1993;306:650.
134. Wakeford R, Tawn EJ, Effects of preconceptional irradiation on mortality and cancer incidence of patients given injections of Thorotrast, *JNCI,* 1995;87:606.
135. Busby C, Bartell R, Schmitz-Feuerhake I, et al., 2010 Reccomendations of the ECRR. The health effects of exposure to low doses of ionizing radiation regulators' edition, Published on behalf of the European Committee on Radiation Risk, Comité Européen sur le Risque de l'Irradiation – Green Audit, 2010:13.
136. Stein RA, Epigenetics and environmental exposures, *J Epidemiol Community Health,* 2012;66(1):8–13.
137. Perera F, Herbstman J, Prenatal environmental exposures, epigenetics, and disease, *Reprod Toxicol,* 2011;31(3):363–73.
138. Kanwal R, Gupta S, Epigenetics and cancer, *J Appl Physiol,* 2010;109:598–605.
139. Esteller M, Epigenetics in cancer, *New Engl J Med,* 2008;358:1148–59.
140. Feinberg AP, Ohlsson R, Henikoff S, The epigenetic progenitor origin of human cancer, *Nat Rev Genet,* 2005;7,21–33.
141. Holliday R, Epigenetics: a historical overview, *Epigenetics,* 2006;1,76–80.
142. Feinberg AP, Vogelstein B, Hypomethylation of ras oncogenes in primary human cancers, *Biochem Biophys Res Comm,* 1983;111:47–54.
143. De La Rosa-Velazquez IA, Rincon-Arano H, Benıtez-Bribiesca L, et al., Epigenetic regulation of the human retinoblastoma tumor suppressor gene promoter by CTCF, *Cancer Res,* 2007;67:2577–85.
144. Fiskus W, Wang Y, Sreekumar A, et al., Combined epigenetic therapy with the histone methyltransferase EZH2 inhibitor 3-deazaneplanocin A and the histone deacetylase inhibitor panobinostat against human AML cells, *Bood,* 2009;114:2733–43.
145. Yoo CB, Jones PA, Epigenetic therapy of cancer: past, present and future, Nat Rev Drug Discov, 2006;5:37–50.
146. Ganesan A, Nolan L, Crabb SJ, et al., Epigenetic therapy: histone acetylation, DNA methylation and anti-cancer drug discovery, *Curr Cancer Drug Targets,* 2009;9:963–81.
147. Verma M, Manne U, Genetic and epigenetic biomarkers in cancer diagnosis and identifying high risk populations, *Crit Rev Oncol Hematol,* 2006;60:9–18.
148. Bock C, Epigenetic biomerkers development, *Epigenomics,* 2009;1:99–110.
149. Gal-Yam EN, Saito Y, Egger G et al., Cancer epigenetics: modifications, screening and therapy, *Ann Rev Med,* 2008;59:267–80.

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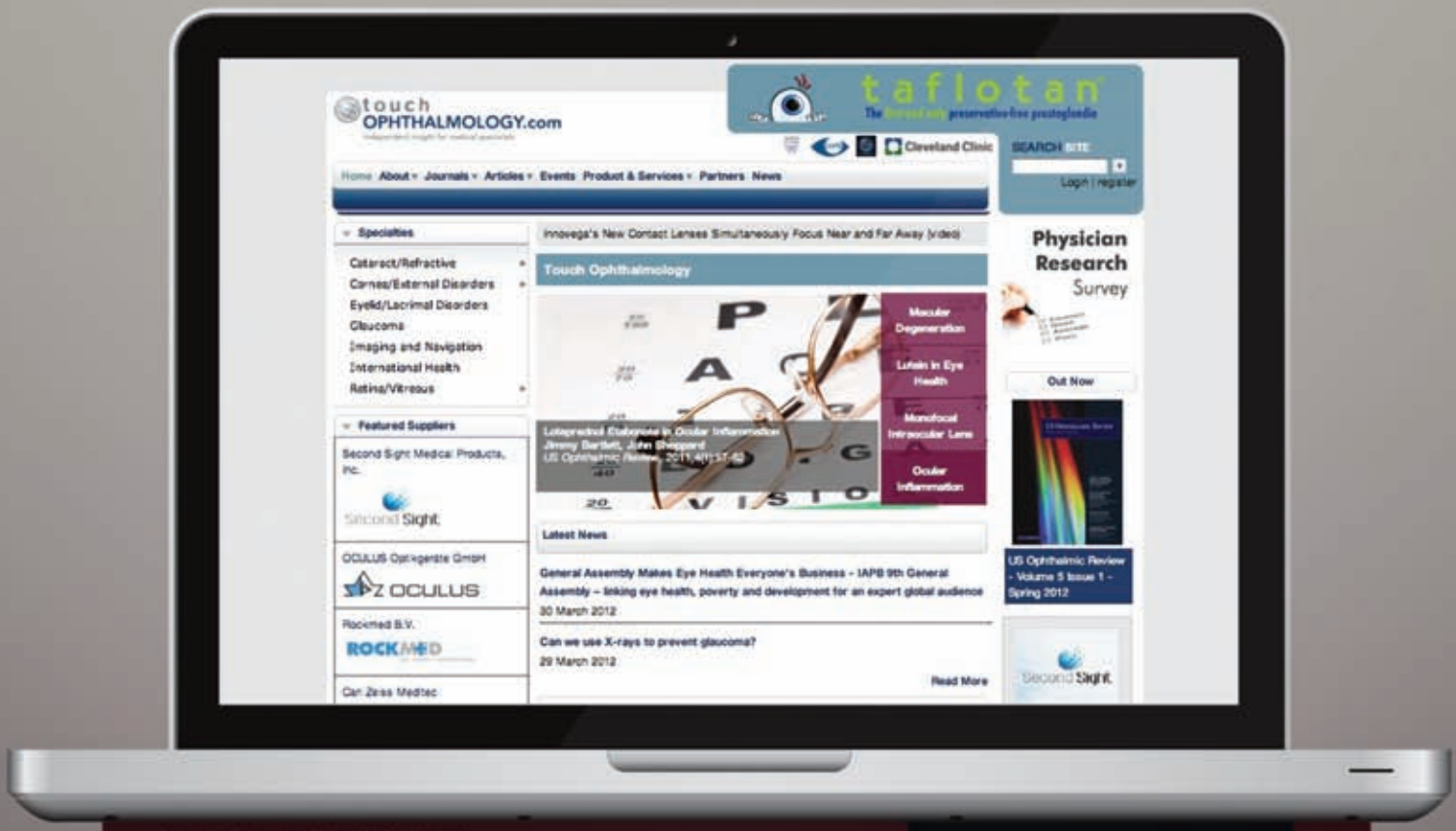

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