6. Borst, M. J. & Ingold, J. A. Metastatic patterns of invasive lobular versus invasive ductal carcinoma of the breast. *Surgery* **114**, 637–641 (1993).
7. Elledge, R. M. *et al. HER2* expression and response to tamoxifen in estrogen receptor-positive breast cancer: a Southwest Oncology Group Study. *Clin. Cancer Res.* **4**, 7–12 (1998).
8. Elston, C. W. *et al.* Causes of inconsistency in diagnosing and classifying intraductal proliferations of the breast. *Eur. J. Cancer* **36**, 1769–1772 (2000).
9. Jones, C. *et al.* Comparative genomic hybridization analysis of myoepithelial carcinoma of the breast. *Lab. Invest.* **80**, 831–836 (2000).
10. Jones, C. *et al.* CGH analysis of ductal carcinoma of the breast with basaloid/myoepithelial cell differentiation. *Br. J. Cancer* **85**, 422–427 (2001).
11. Tsuda, H. *et al.* Large, central acellular zones indicating myoepithelial tumor differentiation in high-grade invasive ductal carcinomas as markers of predisposition to lung and brain metastases. *Am. J. Surg. Pathol.* **24**, 197–202 (2000).
12. Kitahara, O. *et al.* Altered gene expression during colorectal carcinogenesis revealed by cDNA microarrays after laser capture microdissection of tumour tissues and normal epithelia. *Cancer Res.* **61**, 3544–3549 (2001).
13. Sgroi, D. C. *et al. In vivo* gene expression profile analysis of human breast cancer progression. *Cancer Res.* **59**, 5656–5661 (1999).
14. Pollack, J. R. *et al.* Genome-wide analysis of DNA copy-number changes using cDNA microarrays. *Nature Genet.* **23**, 41–46 (1999).
15. Simone, N. L. *et al.* Laser-capture microdissection: opening the microscopic frontier to molecular analysis. *Trends Genet.* **14**, 272–276 (1998).
16. Sapolsky, R. J. *et al.* High-throughput polymorphism screening and genotyping with high-density oligonucleotide arrays. *Genet. Anal.* **14**, 187–192 (1999).
17. Page, M. J. *et al.* Proteomic definition of normal human luminal and myoepithelial breast cells purified from reduction mammoplasties. *Proc. Natl Acad. Sci. USA* **96**, 12589–12594 (1999).
18. Kononen, J. *et al.* Tissue microarrays for high-throughput molecular profiling of tumor specimens. *Nature Med.* **4**, 844–847 (1998).
19. Camp, R. L. *et al.* Validation of tissue microarray technology in breast carcinoma. *Lab. Invest.* **80**, 1943–1949 (2000).
20. Khan, J. *et al.* Classification and diagnostic prediction of cancers using gene expression profiling and artificial neural networks. *Nature Med.* **7**, 673–679 (2001).
21. Hedenfalk, I. *et al.* Gene-expression profiles in hereditary breast cancer. *N. Engl. J. Med.* **344**, 539–548 (2001).

Online links

DATABASES

The following terms in this article are linked online to:

CancerNet: http://cancernet.nci.nih.gov/
breast tumour | colorectal tumour | squamous cell carcinomas | thyroid tumours

LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink/
ABL | BCR | *HER2/NEU* | oestrogen receptor

Medscape DrugInfo:
http://promini.medscape.com/drugdb/search.asp
Herceptin | Gleevec | Tamoxifen

OMIM: http://www.ncbi.nlm.nih.gov/Omim/
multiple endocrine neoplasia

FURTHER INFORMATION

Breast Pathology Index: http://www-medlib.med.utah.edu/WebPath/BRESHTML/BRESTIDX.html

Introduction to Microarray Analysis:
http://www.nhgri.nih.gov/DIR/LCG/15K/HTML/

Laser Capture Microdissection:
http://mecko.nichd.nih.gov/lcm/lcm.htm

NHGRI Introduction to DNA Microarray Technology:
http://industry.ebi.ac.uk/~alan/MicroArray/IntroMicroArrayTalk/index.htm

Stanford University Microarray site:
http://cmgm.stanford.edu/pbrown/array.html

Tumour Pathology: http://www.tumorboard.com/

Access to this interactive links box is free online.

TIMELINE

Two genetic hits (more or less) to cancer

Alfred G. Knudson

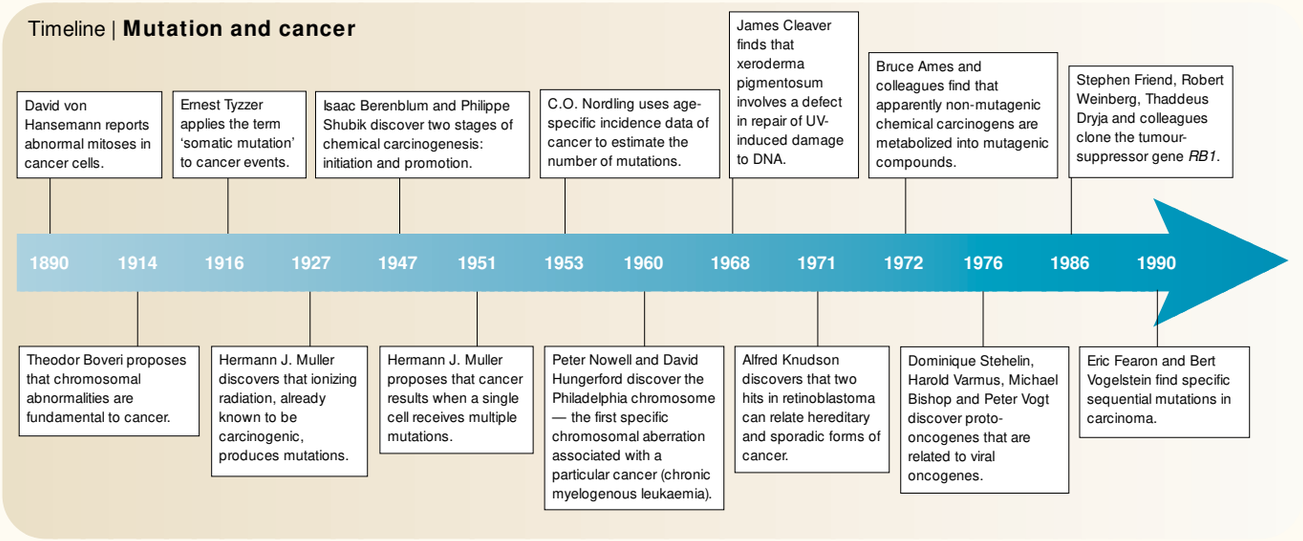
Most cancers have many chromosomal abnormalities, both in number and in structure, whereas some show only a single aberration. In the era before molecular biology, cancer researchers, studying both human and animal cancers, proposed that a small number of events was needed for carcinogenesis. Evidence from the recent molecular era also indicates that cancers can arise from small numbers of events that affect common cell birth and death processes.

We are now very familiar with the concept that cancer occurs as a consequence of several somatic mutations, but how did this concept first arise? The idea that cancer is a genetic disease of somatic cells — proposed by Theodor Boveri in 1914 (REF. 1) — was prompted by previous observations of aberrant mitoses by David von Hansemann[2], and by Boveri's own interest in centrosomes and their abnormalities during development (see TIMELINE). Boveri even suggested some consequences of abnormal chromosome numbers, anticipating the contemporary era of tumour-suppressor genes and oncogenes (BOX 1)[3]. The term 'somatic mutation' was first applied to cancer by Ernest Tyzzer[4], who observed that tumours sequentially transplanted in mice developed an ever-broadening host specificity among recipients from different inbred strains. Concrete support for the genetic concept came from Hermann J. Muller's[5] discovery that ionizing radiation, already known to be carcinogenic, is mutagenic. The long latent period between exposure to such radiation and the appearance of most of the inducible cancers further indicated that more than one mutation per cell must be involved[6]. Subsequently, the high incidence of skin cancer in patients with xeroderma pigmentosum, a condition to which Boveri drew attention[1], was shown to be a consequence of somatic mutations in the presence of a hereditary defect in the repair of ultraviolet-light-induced damage to DNA[7]. Chemical carcinogenesis also fitted into the mutational concept of cancer with the observations of 'initiation' by carcinogens and of 'promotion' by other kinds of chemicals[8], the former being an irreversible change, probably mutation, the latter, a reversible change affecting the growth kinetics of the target cells. But although some initiating chemical carcinogens were found to be mutagenic, others were not; this discrepancy was resolved when Bruce Ames and colleagues discovered that non-mutagenic initiators could be made mutagenic by metabolic activation[9]. Most cancers came to be considered to be initiated by somatic mutation, either induced or spontaneous. The suggestion that more than one event seemed to be required for carcinogenesis then raised a question about their number.

The number of 'hits'

A conspicuous feature of the epidemiology of common cancers is that their incidence increases with age, so the notion of multiple mutations was invoked by way of explanation[10,11]. If r successive mutations occur in some cells at constant rates — $k_1, k_2, \ldots k_r$ per unit time (t), if the size of the target-cell population remains constant, and if cells with an intermediate number of mutations have no growth advantage, the age-specific incidence (I) would be $I = kt^{r-1}$. Therefore, a log–log plot of the relationship would be $\ln I = \ln k + (r-1) \ln t$ — a linear relationship in which the slope would yield $r-1$ (FIG. 1). Many cancers show this relationship, and r has been estimated for numerous cancers; for example, $r = 6$ for colon cancer[10,12]. This, of course, would be the number of rate-limiting events that produce a recognizable cancer. Subsequent events of biological importance for invasion and metastasis would not be included in this number if the sixth event gave a suddenly large growth advantage, producing an obvious tumour.

A possible fallacy in the estimation of r is that the mutation rate might change with time. An obvious example is provided by lung cancer and smoking. Given the mutagenic effect of tobacco smoke, the mutation rate for a particular step in the process would be increased at the onset of smoking. Another case in which r can be incorrectly estimated is a biologically important event that occurs at a higher rate than is usual for mutations that are rate limiting and would



therefore not be counted; inactivation of gene expression by methylation might constitute such an event.

Some cancers do not fit the mathematical model for other reasons. For example, the childhood cancers show a peak incidence in early life because the cells that give rise to tumours attain maximum numbers at that time. Similarly, osteosarcoma has an increasing incidence during adolescence, when the rate of growth of the long bones is highest. Breast-cancer incidence increases more slowly after the menopause, causing a downturn in the log–log plot of age-specific incidence. This is presumably due to a decrease in the number of dividing cells that could give rise to tumours after the menopause.

The notion that there is no growth advantage in intermediate stages is also faulty for many cancers. For example, most colorectal carcinomas arise from adenomatous polyps, the cells of which clearly have a growth advantage that leads to a benign neoplasm. In theory, a log–log plot of age-specific colon cancer incidence in people with familial adenomatous polyposis (FAP) should show a slope that is compatible with one less somatic event (that is, $r = 5$), because these individuals have an inherited mutation (in the *APC* gene) that predisposes them to colorectal cancer. But because polyps already have a growth advantage, r for FAP is 3–4, showing how an intermediate growth advantage can affect the relationship to age[13]. For non-hereditary colon cancer the number of events should be 4–5, rather than 6. Two of these are accounted for by the mutation or loss of the two alleles of the *APC* gene that leads to polyp formation[14,15]. This confounding effect of intermediate growth advantage on the number of hits deducible from incidence curves was already anticipated by Peter Armitage and Richard Doll[16], contributors to the original interpretation[10], in a second paper in which they fitted cancer-incidence curves to a two-mutation curve that took growth advantage into account. In a later model, this advantage was attributed to both an increase in cell birth rate and a decrease in cell death rate[17,18]. So, can we calculate the number of mutations necessary for a tumour to occur, simply from a log–log plot of age-specific incidence? We can conclude that the age-specific incidence for a cancer depends on the mitotic rate of target cells, mutation rates per mitosis, the number of mutational events on the path to detectable cancer and selective processes that occur at each step in the evolution of tumours. Without specific knowledge of these factors, however, the precise number of crucial events cannot be estimated. Are there, then, other means for discovering the number and nature of such events?

Box 1 | Boveri's prediction of oncogenes and tumour-suppressor genes

"…in every normal cell there is a specific arrangement for inhibiting, which allows the process of division to begin only when the inhibition has been overcome by a special stimulus. To assume the presence of definite chromosomes which inhibit division, would harmonize best with my fundamental idea … Cells of tumours with unlimited growth would arise if those 'inhibiting chromosomes' were eliminated … On the other hand, the assumption of the existence of chromosomes which promote division, might satisfy this postulate … cell-division would take place when the action of these chromatin parts … should be strengthened by a stimulus … If three or four such chromosomes meet, the whole number of chromosomes being otherwise normal, then the tendency to rapid proliferation would arise." Boveri (1914)[1].

Cytogenetics and 'one-hit' tumours

One such means has been the cytogenetic examination of cancers with modern techniques. Although most cancers reveal extensive chromosomal instability, which is visible by karyotype analysis, a remarkably contrary discovery was made by Peter Nowell and David Hungerford in 1960 (REF. 19). In the chronic myelogenous leukaemia (CML) cells that they examined, they found the same cytogenetic change: chromosome 22 was too small. They named this chromosome the Philadelphia chromosome (Ph^1), and it was later shown by Janet Rowley to result from a reciprocal translocation between chromosomes 9 and 22 (FIG. 2a)[20]. The subsequent discovery of cellular proto-oncogenes by Dominique Stehelin *et al.*[21] and the demonstration by Robert Weinberg and colleagues[22] of *in vitro* transformation by DNA, paved the way to a mechanistic understanding of how translocations lead to cancer. Still later, following the discovery that the typical 8;14 translocation in Burkitt's lymphoma activated the *MYC* oncogene[23,24], the CML translocation was found to activate the Abelson (*ABL*) oncogene[25–27]. The resulting chimeric ABL protein seems to interfere with regulation of both the cell cycle (increasing cancer-cell birth rate) and apoptosis (decreasing cancer-cell death rate), via its activation of the AKT oncoprotein[28]. CML continues to provide excitement because the increased tyrosine kinase activity of the chimeric *ABL* gene product in the leukaemic cells can be

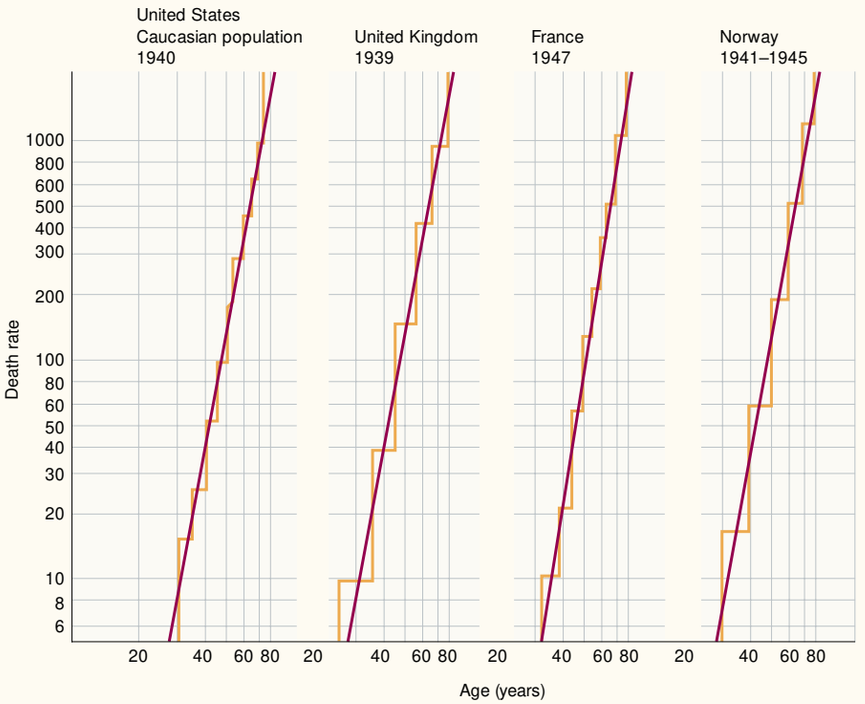


Figure 1 | **Log–log plots of cancer death rates in males (per 100,000) versus age, showing a linear relationship that is consistent throughout the developed world.** (Reproduced, with permission, from REF. 11 ©(1953) Harcourt, Inc.)

inhibited both *in vitro* and *in vivo* by a specific chemical agent (STI-571; Gleevec) (REFS 29,30); the presumptively single abnormality is functionally crucial for the cancer.

Although we cannot be sure that there are no other mutations in CML, below the resolution of cytogenetics, in its early and apparently 'one-hit' chronic phase, CML is strikingly different karyotypically from multihit carcinomas (FIG. 2b). When first diagnosed, CML is relatively benign but, after a few years, an acute blastic crisis ensues in which other chromosomal aberrations are observed; in some cases, the other aberration is acquisition of a second Ph[1], so two copies of the activated chimeric gene produce a more serious effect than one. As many leukaemias, lymphomas and sarcomas are characterized by solitary, specific translocations, an increasingly long list of activated oncogenes has emerged. Furthermore, many of these cancers also acquire other chromosomal aberrations as they progress, so the whole group teaches us that a kind of genomic instability might occur *after* a cancer has resulted from what seems to be a single event, a specific translocation.

Retinoblastoma is a 'two-hit' tumour

Another means for investigating cancer events is the study of hereditary cancers, exemplified here by retinoblastoma. Some cancers occur almost exclusively in children, reflecting their origin from a type of cell that normally differentiates into a different type and ceases to exist in its original form. There is no *a priori* need to hypothesize a large number of mutations in childhood cancers. In fact, some cases are apparent at birth, hardly enough time for many mutational events. Retinoblastoma is such a cancer, arising from fetal retinoblasts that normally differentiate into post-mitotic retinal photoreceptor cells and neurons. Differentiation fails to occur normally in the tumours, and the cells continue to cycle. Ultimately, they spread and metastasize.

Predisposition to retinoblastoma is imparted by a germ-line mutation in approximately 40% of cases in the United States[31]. I was interested in the fact that the germ-line mutation, which is a *de novo* mutation in 80% of the germ-line mutants, is not a sufficient condition for tumorigenesis — some children with an affected parent do not develop a tumour, but later produce an affected child, indicating that they carry the germ-line mutation. Most affected children with an affected parent develop tumours bilaterally, but some do so unilaterally. Approximately 60% of all cases are unilateral in the United States and do not carry a predisposing germ-line mutation. I calculated that the numbers of tumours per heritable case followed a Poisson distribution, with a mean of three. From this, it can be inferred that 5% ($e^{-3} = 0.05$) of carriers of the germ-line mutation would develop no tumour, which fits approximately with observation[31]. The distribution of bilateral cases that have not yet been diagnosed (S) at different ages showed a linear decline on a semilog plot (that is, $\ln S = -kt$, where k is a constant that incorporates the mutation rate and t is time), as expected for a one-hit phenomenon (FIG. 3). From this, I predicated that hereditary retinoblastoma involves two mutations and, knowing that one of these had to be a germ-line mutation, I hypothesized that the other one would be somatic. The unilateral cases with no positive family history, only a minority of whom carry a germ-line mutation, showed a distribution that is consistent with two mutations, so both of these ought to be somatic. The hereditary and nonhereditary forms of the tumour seemed to entail the same number of events — a hypothesis that became known as the 'two-hit hypothesis'. These somatic mutations apparently occur at usual mutation rates. So, in the hereditary cases the somatic (second-event) mutations that would account for the Poisson mean of

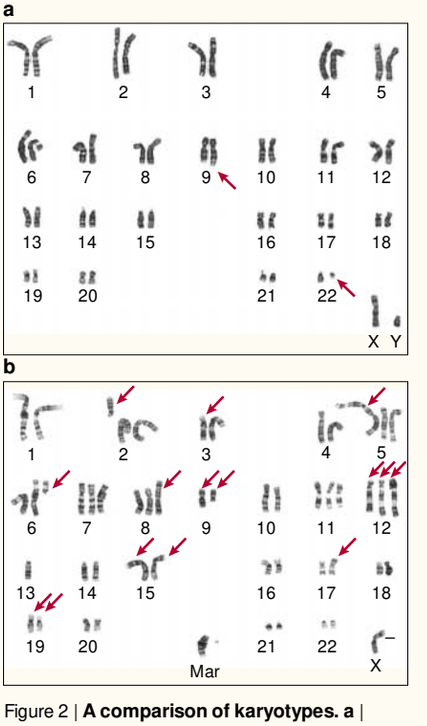


Figure 2 | **A comparison of karyotypes. a** | Chronic myelogenous leukaemia, showing the typical 9;22 translocation and an otherwise normal karyotype. **b** | Non-small-cell carcinoma of the lung, showing abnormalities of both number and structure. The arrows indicate aberrant chromosomes.

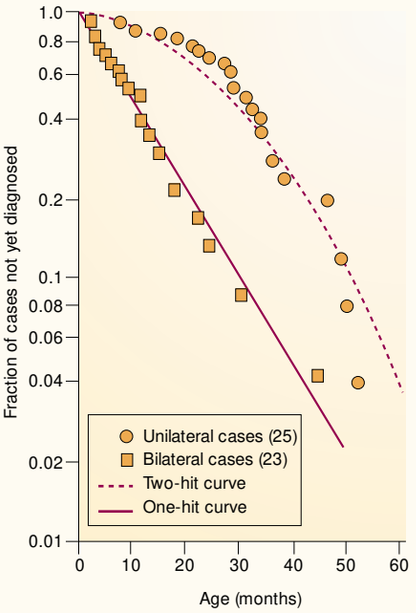


Figure 3 | **One-hit and two-hit curves for retinoblastoma.** These semilog plots of the fraction of 23 bilateral (heritable) cases and 25 unilateral (most expected to be non-heritable) cases that were still not diagnosed at plotted ages (data were analysed retrospectively) show that the bilateral cases match the expected shape of a one-hit curve, whereas the unilateral cases match the shape of a two-hit curve. As the bilateral cases inherit one genetic hit, both heritable and spontaneous retinoblastoma are due to two hits.

three tumours per individual — that is, one somatic mutation in each of three different cells, each leading to a different tumour — are found against the background of the millions of mitoses that are necessary to generate differentiated retinal epithelium from fetal retinoblasts. Our model for retinoblastoma took into account this growth and required a mutation rate of 10^{-6} or less per locus per mitosis[32]. In the nonhereditary cases, the first somatic mutation might be expected to occur at a rate approximately equal to that of the second mutation in the hereditary cases, implying that the retinas of most people contain clones of cells that have sustained one hit, but differentiated before a second hit could occur. Second somatic events in these clones would be expected, at spontaneous mutation rates of 10^{-6} or so, to yield the observed incidence of the nonhereditary form of the tumour, which is 60% of the total birth incidence rate of about 5×10^{-5}, or 3×10^{-5}. There is no need to invoke a high mutation rate for the origin of retinoblastoma, primarily because it arises in a rapidly expanding population of retinoblasts during fetal development of the eye (FIG. 4).

The meaning of two hits

What are the implications of two mutations in tumorigenesis? Are they dominant mutations in two different genes, or recessive mutations in the two alleles of one gene? David Comings and I both favoured the latter notion[33,34]. I later applied the name anti-oncogene to such genes, but they are now known as tumour-suppressor genes, although both terms place them in opposition to oncogenes. I proposed that the second event could be caused by intragenic mutation, whole gene deletion, chromosomal loss by nondisjunction or somatic recombination[35], but evidence was not forthcoming until the application, by Webster Cavenee and colleagues, of DNA restriction fragment length polymorphisms (RFLPs) to the study of cancer[36]. Here again, cytogenetic analysis was vital in uncovering the mechanism behind the two hits in retinoblastoma: a few cases are associated with a germ-line (usually *de novo*) deletion of chromosomal band 13q14 (REFS 37,38). Heterozygosity for linked, but external, markers on chromosomal 13 would be lost with deletion, chromosomal loss or recombination, but not with intragenic mutations. The use of RFLPs supported the conclusion that any of these mechanisms can occur as second events in retinoblastoma. This work provided direct evidence for the identification of *RB1* as a tumour-suppressor gene. This was subsequently shown to be the case following the cloning of the gene[39]; *RB1* became the first tumour-suppressor gene to be characterized. Its protein product is a key regulator of the cell cycle, and hence of the birth rate of cells. Loss of the protein is accompanied by failure of retinoblasts to differentiate normally. Incidentally, the cytogenetic discovery of germ-line aberrations, together with the use of RFLPs, led to the cloning not only of *RB1*, but also of several other hereditary cancer genes, including *WT1*, *NF1*, *NF2* and *APC*.

Back to more than two hits

At about the same time, another gene, *TP53*, was found to have a principal role in controlling the death of tumour cells. Although discovered during the study of the mechanism of transformation by DNA tumour viruses[40,41], it was later shown to be a tumour-suppressor gene[42], and to be mutated[43] in the germ line of persons with Li–Fraumeni syndrome[44] — an hereditary predisposition to several cancers, especially breast cancer. We now know that the gene's protein product, p53, is a multifunctional protein that allows cells to respond appropriately to stress by controlling the cell cycle, DNA repair and apoptosis, but most pertinent to this discussion is its function as an important mediator of apoptosis[45]. *RB1* and *TP53* — or genes that function in their pathways — are inactivated in most cancers, thereby both increasing tumour-cell birth rate and decreasing death rate. Interestingly, loss of *TP53* leads to defective

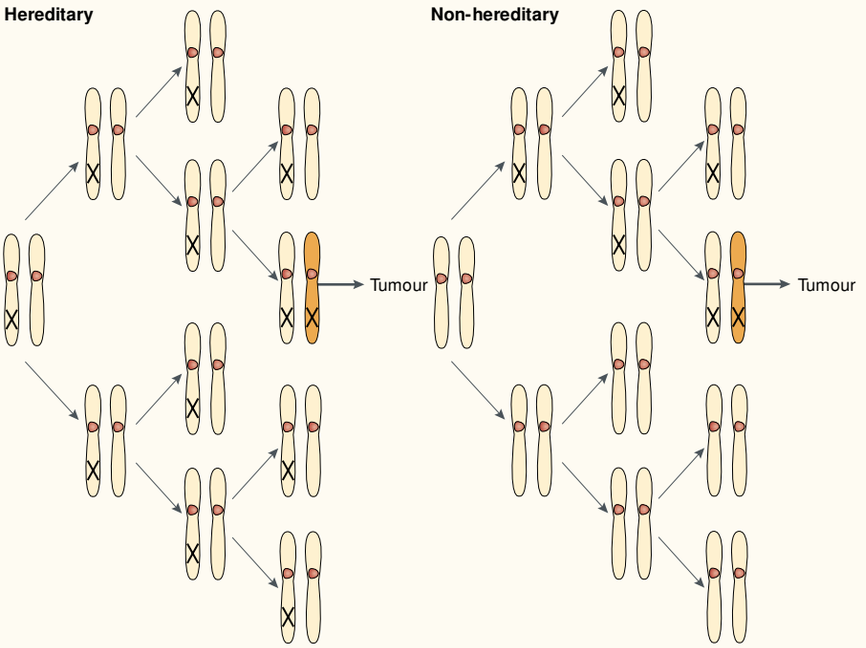


Figure 4 | **Two-hit tumour formation in both hereditary and nonhereditary retinoblastoma.** A 'one-hit' clone is a precursor to the tumour in nonhereditary retinoblastomas, whereas all retinoblasts (indeed, all cells) are one-hit clones in hereditary retinoblastoma.

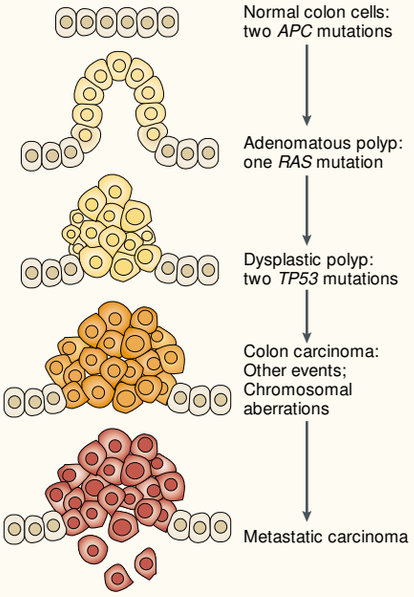


Figure 5 | **A possible five-hit scenario for colorectal cancer, showing the mutational events that correlate with each step in the adenoma–carcinoma sequence.** Based on a model from Fearon and Vogelstein (REF. 47).

centrosome replication and numerous chromosomal abnormalities[46], the feature of cancer that first attracted the notice of von Hansemann and Boveri.

Inactivation of several other cloned tumour-suppressor genes, including the *APC* gene of FAP, is associated with hereditary cancer and with benign precursors of malignant tumours. These benign lesions, usually adenomatous, are all 'two-hit' tumours that are found in large numbers in the target tissues, undergo malignant transformation at low frequency and require other mutations to do so. These genes, including *APC*, seem to inhibit passage through the cell cycle, so their loss or inactivation increases cell birth rate. In many cases, the transition to frank malignancy involves loss or inactivation of *TP53* (REFS 47,48), thereby reducing cell death rate. Mutations and losses of these two genes could account for four events in the pathway to colon cancer.

The well-known 'adenoma–carcinoma' sequence in colorectal cancer has made this disease a popular model for a multihit cancer[47]. Events on the path to cancer include not only mutations in *APC* and *TP53*, but also in one copy of the *RAS* oncogene[47]. This path would seem to involve five mutational events, a number that is quite compatible with David Ashley's estimate of four or five, which, as described earlier, was calculated from a comparison of log–log plots of age-specific colon cancer incidence in normal and FAP persons, long before we knew of the existence of oncogenes or tumour-suppressor genes (FIG. 5). This number of events could occur at normal spontaneous mutation rates — given the number of cell divisions that occur in the colon over many years, and the clonal expansion that occurs because of selection for mutants that have increased growth rates and decreased death rates from apoptosis during cell turnover[49]. However, the transition from polyp to carcinoma has been reported to be associated with occult genomic instability[50,51], as judged by changes in DNA that are not associated with visible karyotypic abnormalities. It seems that DNA lesions are normally repaired by processes, such as recombinational repair, that leave the chromosome intact. When the induction of this repair is compromised, apoptosis should ensue. This process fails in the presence of *TP53* mutations, and florid karyotypic changes emerge abruptly. This is the state of chromosomal instability (CIN)[52].

Although it is true that some cancers show only one or a few chromosomal abnormalities, most are, like colon cancer, very abnormal at diagnosis. The continued growth of such cancers usually leads, in the absence of intervention, to invasion, metastasis and death over a relatively short time; for most cancers, these events are not rate limiting. The idea that a small number of events can lead to cancer might be correct, but at death there might be many more, some of which provide a further growth advantage subject to clonal selection. Centrosome abnormalities, the emergence of chromosomal breakages, fusions and bridges, and widespread heterologous translocations characterize this period in the life of most cancers. This state clearly represents a 'mutator phenotype'[53].

A second kind of genomic instability — mutational microsatellite instability (MIN)[52] — is not associated with CIN. Tumours that occur in people with hereditary nonpolyposis colorectal cancer (HNPCC) have greatly elevated (~1,000- fold) rates of specific locus mutations[54]. The inherited mutation occurs in mismatch repair (MMR) genes, most frequently *MSH2* or *MLH1*. A somatic mutation in, or loss of, the remaining normal allele renders the affected cell homozygously defective for MMR. Especially vulnerable is the *TGFBR2* gene, which encodes a receptor in an important signal transduction pathway[55]. Mutations in this receptor are strongly selective for increased growth rate. The number of other events that are necessary for production of a carcinoma cell in HNPCC has not been determined. The cells with homozygous mutations in MMR genes clearly have a 'mutator phenotype', even though they do not show CIN. What CIN and MIN seem to have in common is the ability to increase the rate of transit along the path to clinical cancer.

The view ahead

The genetics of cancer has passed from infancy to maturity in the past century and has brought us to a dazzling, often confusing, view. Cancer cells themselves experience birth, development and death (too often with the patient). In some, karyotypic changes are few, whereas in others there is a bewildering array of abnormalities. Consideration of cancers from many perspectives raises the possibility that the crucial changes on the initiating path to all cancers are few, affect both birth and death processes, and are strongly selected for. In tumours with a single genetic defect, a solitary oncogenic translocation (as seems to be the case in chronic-phase CML), the prospect of developing a successfully targeted therapeutic agent promises to be the greatest. By contrast, developing therapies for the 'multi-hit' tumours will be more challenging, as one agent acting on one target might not be sufficient. On the other hand, the time intervals between multiple hits might be windows of opportunity for preventive agents, in which transition to the next step (such as the second hit in generating the colonic adenomatous polyp) could be delayed or prevented.

Fox Chase Cancer Center, 7701 Burholme Avenue, Philadelphia, Pennsylvania 19111, USA.
e-mail: ag_knudson@fccc.edu

1. Boveri, T. *Zur Frage der Entstehung Maligner Tumoren* (Gustav Fischer, Jena); English translation *The Origin of Malignant Tumors* by Boveri, M. (Williams and Wilkins, Baltimore, 1929, 1914).
2. von Hansemann, D. Über asymmetrische Zellteilung in Epithelkrebsen und deren biologische Bedeutung. *Virchow's Arch. Path. Anat.* **119**, 299–326 (1890).
3. Balmain, A. Cancer genetics: from Boveri and Mendel to microarrays. *Nature Rev. Cancer* **1**, 77–80 (2001).
4. Tyzzer, E. E. Tumor immunity. *J. Cancer Res.* **1**, 125–156 (1916).
5. Muller, H. J. Artificial transmutation of the gene. *Science* **46**, 84–87 (1927).
6. Muller, H. J. Radiation damage to the genetic material. *Sci. Progress* **7**, 93–165, 481–493 (1951).
7. Cleaver, J. E. Defective repair replication of DNA in xeroderma pigmentosum. *Nature* **218**, 652–656 (1968).
8. Berenblum, I. & Shubik, P. A new, quantitative, approach to the study of the stages of chemical carcinogenesis in the mouse's skin. *Br. J. Cancer* **1**, 383–391 (1947).
9. Ames, B. N., Sims, P. & Grover, P. L. Epoxides of carcinogenic polycyclic hydrocarbons are frameshift mutagens. *Science* **176**, 47–49 (1972).
10. Armitage, P. & Doll, R. The age distribution of cancer and a multi-stage theory of carcinogenesis. *Br. J. Cancer* **8**, 1–12 (1954).
11. Nordling, C. E. A new theory on the cancer-inducing mechanism. *Br. J. Cancer* **6**, 68–72 (1953).
12. Ashley, D. J. B. The two 'hit' and multiple 'hit' theories of carcinogenesis. *Br. J. Cancer* **23**, 313–328 (1969).
13. Ashley, D. J. B. Colonic cancer arising in polyposis coli. *J. Med. Genet.* **6**, 376–378 (1969).

14. Ichii, S. *et al.* Inactivation of both *APC* alleles in an early stage of colon adenomas in a patient with familial adenomatous polyposis (FAP). *Hum. Mol. Genet.* **1**, 387–390 (1992).
15. Nishisho, I. *et al.* Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. *Science* **253**, 665–669 (1991).
16. Armitage, P. & Doll, R. A two-stage theory of carcinogenesis in relation to the age distribution of human cancer. *Br. J. Cancer* **11**, 161–169 (1957).
17. Moolgavkar, S. H. & Venzon, D. J. Two-event model for carcinogenesis: incidence curves for childhood and adult tumors. *Mater. Biosci.* **47**, 55–77 (1979).
18. Moolgavkar, S. H. & Knudson, A. G. Mutation and cancer: a model for human carcinogenesis. *J. Natl Cancer Inst.* **66**, 1037–1052 (1981).
19. Nowell, P. C. & Hungerford, D. A. A minute chromosome in human chronic granulocytic leukemia. *Science* **132**, 1497 (1960).
20. Rowley, J. D. A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. *Nature* **243**, 290–293 (1973).
21. Stehelin, D., Varmus, H. E., Bishop, J. M. & Vogt, P. K. DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA. *Nature* **260**, 170–173 (1976).
22. Shih, C., Shilo, B. Z., Goldfarb, M. P., Dannenberg, A. & Weinberg, R. A. Passage of phenotypes of chemically transformed cells via transfection of DNA and chromatin. *Proc. Natl Acad. Sci. USA* **76**, 5714–5718 (1979).
23. Dalla-Favera, R. *et al.* Human c-*MYC* onc gene is located on the region of chromosome 8 that is translocated in Burkitt lymphoma cells. *Proc. Natl Acad. Sci. USA* **79**, 7824–7827 (1982).
24. Taub, R. *et al.* Translocation of the c-*myc* gene into the immunoglobulin heavy chain locus in human Burkitt lymphoma and murine plasmacytoma cells. *Proc. Natl Acad. Sci. USA* **79**, 7837–7841 (1982).
25. Konopka, J. B., Watanabe, S. M., Singer, J. W., Collins, S. J. & Witte, O. N. Cell lines and clinical isolates derived from Ph1-positive chronic myelogenous leukemia patients express c-ABL proteins with a common structural alteration. *Proc. Natl Acad. Sci. USA* **82**, 1810–1814 (1985).
26. Shtivelman, E., Lifshitz, B., Gale, R. P. & Canaani, E. Fused transcript of *ABL* and *BCR* genes in chronic myelogenous leukaemia. *Nature* **315**, 550–554 (1985).
27. Stam, K. *et al.* Evidence of a new chimeric *BCR*/c-*ABL* mRNA in patients with chronic myelocytic leukemia and the Philadelphia chromosome. *N. Engl. J. Med.* **313**, 1429–1433 (1985).
28. Skorski, T. *et al.* Transformation of hematopoietic cells by BCR/ABL requires activation of a PI-3k/AKT-dependent pathway. *EMBO J.* **16**, 6151–6161 (1997).
29. Druker, B. J. *et al.* Effects of a selective inhibitor of the ABL tyrosine kinase on the growth of BCR–ABL positive cells. *Nature Med.* **2**, 561–566 (1996).
30. Druker, B. J. *et al.* Activity of a specific inhibitor of the BCR–ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. *N. Engl. J. Med.* **344**, 1038–1042 (2001).
31. Knudson, A. G. Mutation and cancer: statistical study of retinoblastoma. *Proc. Natl Acad. Sci. USA* **68**, 820–823 (1971).
32. Hethcote, H. W. & Knudson, A. G. Model for the incidence of embryonal cancers: application to retinoblastoma. *Proc. Natl Acad. Sci. USA* **75**, 2453–2457 (1978).
33. Comings, D. E. A general theory of carcinogenesis. *Proc. Natl Acad. Sci. USA* **70**, 3324–3328 (1973).
34. Knudson, A. G. Mutation and human cancer. *Adv. Cancer Res.* **17**, 317–352 (1973).
35. Knudson, A. G. Retinoblastoma: a prototypic hereditary neoplasm. *Semin. Oncol.* **5**, 57–60 (1978).
36. Cavenee, W. K. *et al.* Expression of recessive alleles by chromosomal mechanisms in retinoblastoma. *Nature* **305**, 779–784 (1983).
37. Francke, U. & Kung, F. Sporadic bilateral retinoblastoma and 13q- chromosomal deletion. *Med. Pediatr. Oncol.* **2**, 379–385 (1976).
38. Knudson, A. G., Jr, Meadows, A. T., Nichols, W. W. & Hill, R. Chromosomal deletion and retinoblastoma. *N. Engl. J. Med.* **295**, 1120–1123 (1976).
39. Friend, S. H. *et al.* A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. *Nature* **323**, 643–646 (1986).
40. Lane, D. P. & Crawford, L. V. T antigen is bound to a host protein in SV40-transformed cells. *Nature* **278**, 261–263 (1979).
41. Linzer, D. I. & Levine, A. J. Characterization of a 54K dalton cellular SV40 tumor antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells. *Cell* **17**, 43–52 (1979).
42. Finlay, C. A., Hinds, P. W. & Levine, A. J. The p53 proto-oncogene can act as a suppressor of transformation. *Cell* **57**, 1083–1093 (1989).
43. Malkin, D. *et al.* Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science* **250**, 1233–1238 (1990).
44. Li, F. P. & Fraumeni, J. F. Soft-tissue sarcomas, breast cancer, and other neoplasms. A familial syndrome? *Ann. Intern. Med.* **71**, 747–752 (1969).
45. Yonish-Rouach, E. *et al.* Wild-type p53 induces apoptosis of myeloid leukaemic cells that is inhibited by interleukin-6. *Nature* **352**, 345–347 (1991).
46. Fukasawa, K., Choi, T., Kuriyama, R., Rulong, S. & Vande Woude, G. F. Abnormal centrosome amplification in the absence of p53. *Science* **271**, 1744–1747 (1996).
47. Fearon, E. R. & Vogelstein, B. A genetic model for colorectal tumorigenesis. *Cell* **61**, 759–767 (1990).
48. Kikuchi-Yanoshita, R. *et al.* Genetic changes of both p53 alleles associated with the conversion from colorectal adenoma to early carcinoma in familial adenomatous polyposis and non-familial adenomatous polyposis patients. *Cancer Res.* **52**, 3965–3971 (1992).
49. Tomlinson, I. & Bodmer, W. Selection, the mutation rate and cancer: ensuring that the tail does not wag the dog. *Nature Med.* **5**, 11–12 (1999).
50. Shih, I. M. *et al.* Evidence that genetic instability occurs at an early stage of colorectal tumorigenesis. *Cancer Res.* **61**, 818–822 (2001).
51. Stoler, D. L. *et al.* The onset and extent of genomic instability in sporadic colorectal tumor progression. *Proc. Natl Acad. Sci. USA* **96**, 15121–15126 (1999).
52. Lengauer, C., Kinzler, K. W. & Vogelstein, B. Genetic instabilities in human cancers. *Nature* **396**, 643–649 (1998).
53. Loeb, L. A. Mutator phenotype may be required for multistage carcinogenesis. *Cancer Res.* **51**, 3075–3079 (1991).
54. Bhattacharyya, N. P., Skandalis, A., Ganesh, A., Groden, J. & Meuth, M. Mutator phenotypes in human colorectal carcinoma cell lines. *Proc. Natl Acad. Sci. USA* **91**, 6319–6323 (1994).
55. Markowitz, S. *et al.* Inactivation of the type II TGF-β receptor in colon cancer cells with microsatellite instability. *Science* **268**, 1336–1338 (1995).

Online links

DATABASES

The following terms in this article are linked online to:

CancerNet: http://cancernet.nci.nih.gov/
breast cancer | Burkitt's lymphoma | chronic myelogenous leukaemia | colorectal carcinomas | osteosarcoma | retinoblastoma

LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink/
ABL | AKT | *APC* | *MLH1* | *MSH2* | *MYC* | *NF1* | *NF2* | *HRAS* | *RB1* | *TGFBR2* | *TP53* | *WT1*

Medscape DrugInfo:
http://promini.medscape.com/drugdb/search.asp
Gleevec

OMIM: http://www.ncbi.nlm.nih.gov/Omim/
familial adenomatous polyposis | hereditary non-polyposis colorectal cancer | Li–Fraumeni syndrome

Access to this interactive links box is free online.

OPINION

Actin' up: RHOB in cancer and apoptosis

George C. Prendergast

RHOB is a small GTPase that regulates actin organization and vesicle transport. It is required for signalling apoptosis in transformed cells that are exposed to farnesyltransferase inhibitors, DNA-damaging agents or taxol. Genetic analysis in mice indicates that RhoB is dispensable for normal cell physiology, but that it has a suppressor or negative modifier function in stress-associated processes, including cancer.

RHO proteins are receiving increasing attention from cancer researchers owing to evidence that they modulate the proliferation, survival, invasion and angiogenic capacity of cancer cells. This family of actin regulatory small GTPases (BOX 1) is not mutated in cancer. However, their altered expression or activity might be crucial to cancer progression and therapeutic responses.

Recent advances indicate that RHOB is a specialized activator of apoptosis in transformed cells. Through a gain-of-function mechanism, RHOB has an important role in mediating the cellular response to farnesyltransferase inhibitors (FTIs). These experimental therapeutics are widely known for their selective effects on neoplastically transformed cells. Although some questions remain about exactly how RHOB alteration fits into the FTI response, many of the biological effects of FTI treatment have been linked to RHOB. Of particular interest, evidence indicates that RHOB is a crucial target for FTI-induced apoptosis. Recently, this role was extended with the finding that RHOB is required for the apoptotic response of transformed cells to DNA damage or TAXOL. Genetic analysis in mice indicates that RhoB is dispensable for normal cell physiology, but that it limits cancer susceptibility and modifies growth-factor and adhesion signalling in transformed cells. What are RHOB's effector mechanisms, and how might they promote apoptosis?

Unique features of RHOB

RHO proteins, which are themselves a subset of the RAS superfamily of isoprenylated small GTPases, can be further divided into subgroups of RHO, RAC and CDC42 proteins. These regulate a number of cellular

Databases

CancerNet

Breast cancer
http://cancernet.nci.nih.gov/cgi-bin/srchcgi.exe?TYPE=search&ZUI=208_00013H&DBID=pdq&SFMT=pdq_statement/1/0/0&PASSTHRU=:ip:194.129.50.189::srchform:PDQ:

Burkitt's lymphoma
http://cancernet.nci.nih.gov/cgi-bin/srchcgi.exe?TYPE=search&ZUI=208_00066H&DBID=pdq&SFMT=pdq_statement/1/0/0&PASSTHRU=:ip:194.129.50.189::srchform:PDQ:

Chronic myelogenous leukaemia
http://cancernet.nci.nih.gov/cgi-bin/srchcgi.exe?TYPE=search&ZUI=208_01031H&DBID=pdq&SFMT=pdq_statement/1/0/0&PASSTHRU=:ip:194.129.50.189::srchform:PDQ:

Colorectal carcinomas
http://cancernet.nci.nih.gov/cgi-bin/srchcgi.exe?TYPE=search&ZUI=208_00008H&DBID=pdq&SFMT=pdq_statement/1/0/0&PASSTHRU=:ip:194.129.50.189::srchform:PDQ:

Osteosarcoma
http://cancernet.nci.nih.gov/cgi-bin/srchcgi.exe?TYPE=search&ZUI=208_00008H&DBID=pdq&SFMT=pdq_statement/1/0/0&PASSTHRU=:ip:194.129.50.189::srchform:PDQ:

retinoblastoma
http://cancernet.nci.nih.gov/cgi-bin/srchcgi.exe?TYPE=search&ZUI=208_00993H&DBID=pdq&SFMT=pdq_statement/1/0/0&PASSTHRU=:ip:194.129.50.189::srchform:PDQ:

LocusLink

ABL
http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=25

AKT
http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=207

APC
http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=324

MLH1
http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=4292

MSH2
http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=4436

MYC
http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=4609

NF1
http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=4763

NF2
http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=4771

RAS
http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=3265

RB1
http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=5925

TGFBR2
http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=7048

TP53
http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=7157

WT1
http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=7490

Medscape DrugInfo

Gleevec
http://promini.medscape.com/drugdb/drug_uses_dosage.asp?DrugCode=1%2D22096&DrugName=GLEEVEC+ORAL&DrugType=1

OMIM

Familial adenomatous polyposis
http://www.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?175100

Hereditary non-polyposis colorectal cancer
http://www.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?120435

Li–Fraumeni syndrome
http://www.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?151623

Biography

Alfred G. Knudson obtained his M.D. from Columbia University, and his Ph.D. from Caltech. He is a native Californian, and is trained in paediatrics and has treated children with cancer at the City of Hope National Medical Center, where he wrote the book *Genetics and Disease* and became interested in viral and genetic theories of cancer. Later, at M. D. Anderson Cancer Center, he proposed his 'two-hit hypothesis' for retinoblastoma. At the Fox Chase Cancer Center, where he is a Senior Member, he has studied hereditary cancer in animals and currently pursues the investigation of prevention in hereditary cancer in humans. He has also written on paediatric cancer with his wife, Dr Anna T. Meadows.