TIMELINE

# Cancer genetics: from Boveri and Mendel to microarrays

*Allan Balmain*

The human genome has now been sequenced, a century after the re-discovery of Mendel's Laws, and the publication of Theodor Boveri's chromosomal theory of heredity. Tracing the historical landmarks of cancer genetics from these early days to the present time not only gives us an appreciation of how far we have come, but also emphasizes the challenges that we face if we are to unravel the genetic basis of hereditary and sporadic cancers in the next century.

Theodor Boveri is one of the towering figures of twentieth-century genetics (FIG. 1), as he was the first to provide a mechanistic basis for the transmission of traits that were proposed by Mendel[1]. Boveri died in 1915, but, unlike Mendel, did not have to wait 50 years to receive recognition from his peers. Two of the significant contemporary figures in developmental biology — E. B. Wilson and Hans Spemann — dedicated their text books to him, and Wilson wrote a piece specifically on Boveri that included the statement: "Boveri stood without a rival among the biologists of his generation; and his writings will long endure as classical models …"[2].

Boveri's work on the fertilization of sea-urchin eggs by two sperm instead of one showed that distribution of unequal numbers of chromosomes to the daughter cells gives rise to specific characteristics that depend on the random combinations of chromosomes that they inherit (FIG. 2). Some daughter cells survive but develop abnormally, whereas others have a genetic imbalance that is too severe and so death ensues. This work convinced Boveri that the individual chromosomes carry different information, a thesis summarized in his chromosomal theory of heredity in 1902–1904 (REFS 3–5).

The association between the abnormal growth of sea-urchin eggs that carry the 'wrong' chromosomal complement and the unrestricted growth of tumours did not escape Boveri's notice and, in his study of sea-urchin chromosomes[3], he suggested that tumours might arise as a consequence of abnormal segregation of chromosomes to daughter cells. This hypothesis was developed and extended in 1914, in his celebrated *Zur Frage der Entstehung Maligner Tumoren* ('*The Origin of Malignant Tumours*')[6]. He postulated that tumour growth is based on '…a particular, incorrect chromosome combination which is the cause of the abnormal growth characteristics passed on to daughter cells…'. In addition to the experimental observations and their insightful interpretations, Boveri made several predictions that, in retrospect, are chillingly accurate. Many concepts that are now commonly accepted were foreshadowed by Boveri, including cell-cycle checkpoints, oncogenes and tumour-suppressor genes, tumour predisposition, and the relationship between genetic instability and cancer (BOX 1). It is a sobering thought that the experimental proof of many of these predictions became the cornerstone of cancer research over the next 85 years (see TIMELINE).

### Proto-oncogenes and cancer

Boveri postulated the existence of 'growth-stimulatory chromosomes' and, furthermore, that the unlimited growth of malignant tumour cells is attributable to a permanent increase in the number of these growth-promoting chromosomes. The concept of the gene had, of course, not been developed at that time, but if we substitute the word 'gene' for 'chromosome', this vision clearly predicted the Nobel-prize-winning discovery of cellular proto-oncogenes by Harold Varmus and Mike Bishop in the 1970s — that genes that are present in all 'normal' cells can become deregulated, amplified or overexpressed and contribute to malignancy[7,8]. This ground-breaking discovery was made through the study of RNA tumour viruses, some of which had captured cellular genes that, when expressed at high level or in mutant form in normal cells, made these cells adopt the characteristics of rapid, uncontrolled growth that are typical of many tumours. The discovery of these genes

Figure 1 | **A portrait of Theodor Boveri.** (Reproduced from Baltzer, F. *Theodor Boveri. Wissenschaftliche Verlagsgesellschaft*. Stuttgart, Germany (1962). Courtesy of Peter Wolbert, The University of Würzburg.)

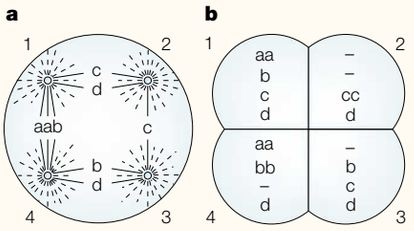



Figure 2 | **Multiple cell poles cause unequal segregation of chromosomes. a** | Boveri showed that fertilization of sea-urchin eggs by two sperm results in multiple cell poles. Individual chromosomes then attach to different combinations of poles — for example, one copy of chromosome c is attached to poles 1 and 2, and one copy is attached to poles 2 and 3. **b** | Chromosomes are segregated to the four poles at cell division, leaving some cells with too many copies of the chromosomes and some with too few — for example, cell 2 has two copies of chromosome c and cell 4 has none.

and the clarification of their roles in the normal processes of growth control, differentiation and development has had a significant impact on our understanding of cellular function, in addition to providing us with an array of targets for the development of new cancer therapies.

The development of cytogenetic techniques was crucial in developing our understanding of the chromosome aberrations that were visualized by Boveri under the microscope. The history of the 'Philadelphia chromosome', a fusion of two chromosome fragments that is detected in the blood cells of patients with chronic myeloid leukaemia (CML), illustrates how therapeutic drug development can arise from an understanding of the genetic changes in cancer cells. The Philadelphia chromosome was initially discovered in 1960 (REF. 9); it took more than a decade to identify the chromosomes involved in the translocation[10], a further decade to find the gene that was activated as a consequence of this change[11], and almost two more decades to develop a drug that is targeted specifically at the activated gene product[12]. Many other cytogenetic studies, predominantly of leukaemias (reviewed in REF. 13), identified a series of specific chromosomal changes that are associated with malignancy, some of which might yield to the same therapeutic strategy as that found for the product of the Philadelphia chromosome translocation.

A revolutionary series of experiments involving DNA transfection provided the first real demonstration of a causal role for genetic alterations in cell transformation. These studies had an electrifying effect on the field of cancer genetics, as they showed that a specific change in DNA could, when transferred in the form of whole genome DNA into an otherwise fairly normal cell, confer at least some of the properties of malignancy on that cell[14]. Further studies on human tumours identified the causative change as a point mutation in a single gene — one of the members of the RAS proto-oncogene family (*HRAS*)[15–17]. Mechanistic studies in animal models showed that this same gene is consistently activated in specific animal models of cancer[18], and that they are caused by exposure to particular carcinogens[19–20]. These studies provided the first direct link between mutagen exposure and changes in target genes that are involved in causing malignancy.

### Tumour-suppressor genes

If the 1970s and early 1980s were the era of RNA tumour viruses and oncogenes, the subsequent decade was dominated by tumour-suppressor genes. These were also predicted by Boveri, who foresaw that 'inhibitory chromosomes' (*teilungshemmende Chromosomen*) would be physically removed by malignant tumours. He postulated that inhibitory chromosomes formed part of a mechanism that keeps normal cells in check, until a specific extracellular stimulus relieves the inhibition and allows cell division to proceed. The prototype tumour-suppressor gene, known as the retinoblastoma or *RB* gene[21], fulfilled these criteria as it inhibits cell-cycle progression at the G1/S boundary and responds to external growth-factor stimulation by becoming phosphorylated at specific sites, which, in turn, removes the inhibitory influence and allows passage through the checkpoint[22–23]. The concept of inherited predisposition and homozygous inactivation of a tumour-suppressor gene was also noted by Boveri. He predicted that cancer predisposition could be attributed to the inheritance of chromosomes (genes) that have "weaker resistance against the action of factors that stimulate cell division"[6]. Furthermore, for a tumour to develop, "the homologous elements of both chromosomes have to be similarly weakened"[6], leading to a chromosomal explanation of the increased incidence of cancer in the progeny of consanguineous marriages.

> "Boveri stood without a rival among the biologists of his generation; and his writings will long endure as classical models…"

In 1971, Knudson carried out an epidemiological study of retinoblastoma development in children. The results echoed some of Boveri's predictions, but allowed the formulation of a mathematical model that was subject to experimental testing. He postulated that 'two hits' are required for the complete inactivation of a

Box 1 | **Boveri's predictions**

- Cell-cycle checkpoints (*Hemmungseinrichtung*: inhibitory mechanism) that would allow cell division only when a specific external stimulus is experienced by the cell.
- Tumour-suppressor genes (*Teilungshemmende Chromosomen*), the effects of which can be overcome by external signals, and which are physically lost in progressively growing tumours.
- Oncogenes (*Teilungsfoerdernde Chromosomen*) that become amplified (*im permanenten Übergewicht*) during tumour development.
- Tumour progression from benign to malignant, involving sequential changes of increased growth-stimulatory chromosomes and loss of growth-inhibitory chromosomes.
- The clonal origin of tumours.
- Genetic mosaicism.
- Cancer predisposition through inheritance of chromosomes (genes) that are less able to suppress malignancy.
- Cancer predisposition through inheritance of genes that cause aberrant mitoses.
- Inheritance of the same 'weak chromosome' from both parents leads to homozygosity for the defective chromosome and, consequently, to high-penetrance cancer syndromes — for example, xeroderma pigmentosum.
- The role of wounding and inflammation in tumour promotion.
- Loss of cell adhesion in metastasis.
- Sensitivity of malignant cells to radiation therapy.

tumour-suppressor gene[24], suggesting that cancer predisposition results from inheritance of a specific mutation in a suppressor gene, but that the development of tumours requires subsequent somatic alterations that result in loss of the wild-type copy of the same gene. The tools that are necessary to test the Knudson hypothesis at the molecular level, as well as to detect the chromosomal changes observed by Boveri, were provided by Cavenee and colleagues[25]. They devised methods for tracking the parental origin of particular alleles, and following their subsequent fate during tumorigenesis through loss of heterozygosity (LOH) resulting from somatic deletions or recombinations. These tools were exploited in the mapping[25] and subsequent cloning of *RB*[21], and also by several other groups to identify the 'high-penetrance' genes that are responsible for familial colon and breast cancers[26–30]. The importance of these discoveries lies not only in the identification of the genes, but also in the elucidation of the growth-control pathways in which they operate, which provide a plethora of previously unsuspected diagnostic and therapeutic drug targets.

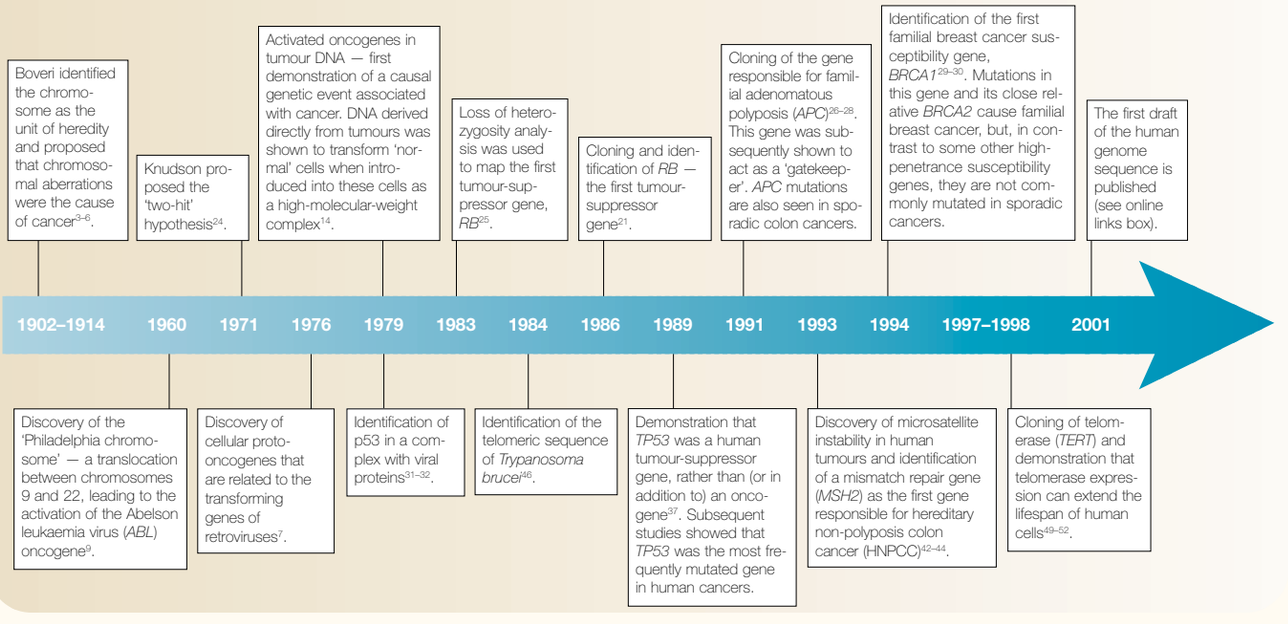
The *TP53* tumour-suppressor gene occupies another special niche in the history of cancer genetics. p53 was first identified in a complex with SV40 T antigen, a protein produced by a DNA tumour virus[30–31], and was initially assumed to act exclusively as an oncogene. The first indications that the story might not be quite so simple came from studies of mouse and human leukaemia cell lines in which *Trp53* and *TP53*, respectively, had rearrangements that led to loss of function, rather than activation[33–35]. In addition, the concept that viral oncoproteins transform cells by binding and inactivating tumour-suppressor proteins was clearly shown for the adenoviral E1A–RB protein interaction[22], raising the possibility that the interaction between p53 and the SV40 large T antigen could also lead to loss of p53 function. Nevertheless, the prevailing view of p53 as an oncogene persisted until LOH evidence from human tumour analysis pinpointed *TP53* within a region that was consistently deleted in tumours[36,37]. Sequencing and functional studies, which identified inactivating or loss-of-function mutations in *TP53*, confirmed its role as a *bona fide* tumour-suppressor gene in agreement with the Knudson two-hit proposal[38]. This work set the stage for an explosion of research on the pathways by which this protein monitors DNA damage in humans and other organisms, and regulates cell growth, cell death and tumorigenesis[39]. Interestingly, *TP53*, like *RB*, exists in mutant heritable forms in the germ line, and contributes to familial cancers when the remaining wild-type allele is lost by somatic genetic alterations[40].

## Genetic instability and cancer

The concept of genetic (chromosomal) instability, which was originally proposed by Boveri as a cause of abnormal growth and cancer, has been confirmed and extended by many investigators over the past two decades. In addition to aneuploidy, caused by uncontrolled segregation of chromosomes to daughter cells, altered stability at the nucleotide level also has an important role. Loeb and colleagues proposed that it would be difficult for an aspiring tumour cell to acquire the number of mutations that are necessary for development of malignancy during the lifetime of the host, and that this conundrum could be solved by postulating the existence of 'mutator' genes[41]. These were predicted to be genes that increase the rate of mutation within tumour cells when they themselves are mutated, allowing the cells to reach the hit rate that is required to eliminate all of the controls exerted by normal checkpoints.

Accordingly, the study of familial cancers has again provided answers, with the discovery of germ-line mutations in genes that affect DNA repair and lead to hereditary non-polyposis colorectal cancer (HNPCC). Defects in genes that control genetic stability at the level of short repeat sequences, rather than at the chromosome level, were first noticed in studies of non-familial or sporadic cancers[42,43], and some of the causative genetic changes were identified in samples from individuals with HNPCC[44]. These and many other studies indicated that the genome is a database of information that is constantly monitored for both large- and small-scale defects. Any deficiencies in normal cells are coupled to efficient mechanisms for repair, or, in certain circumstances, to cell death. The *TP53* tumour-suppressor

Timeline | **Genetic landmarks in cancer research**



gene is one of many that are involved in cell death; consequently, mutations that cause loss of function of these genes lead to an increase in survival of tumour cells[45].

### Telomeres, crisis and cancer

No discussion of the views of Boveri on chromosome instability and cancer would be complete without mentioning the association of telomere crisis with events that lead to tumorigenesis. Telomeres consist of a series of small repeat sequences at the ends of chromosomes that act as caps, protecting them from degradation during cell growth and differentiation[46]. Cell division results in the gradual shortening of telomeres[47], eventually resulting in crisis when the chromosome ends become dysfunctional. This rings an alarm bell that results in cell death by a mechanism that involves activation of p53-mediated apoptosis[48]. Tumours circumvent this fate both by inactivating the cell-death pathway, and by switching on telomerase — an enzyme that helps to maintain telomere length — which allows them to acquire the capacity for infinite cell division[49–52]. These observations, made only within the very recent past, show the uncanny foresight of Boveri in his analysis of the genetic basis of chromosome stability. Although the concept of telomeres did not exist in 1914, the 'weakness' that Boveri discusses in the following passage could be interpreted as the progressive change in telomere length that only becomes manifest after many cell divisions. This telomere shortening leads to crisis, chromosome end-to-end fusions and genetic instability[48]: "For unknown reasons, a 'weakness' may occur in specific chromosomes with respect to control of mitosis that at first remains latent and thus is transmitted to a large number of daughter cells … With the beginning of senescence, perhaps this latent weakness becomes manifest in the failure of mitotic control in such a way that when cell division occurs, there is a possibility of generating daughter cells with recurrent genetic abnormalities."

### Susceptibility to sporadic cancer

It is clear from the above discussion that familial cases of cancer have been more important in the development of our understanding of the genetic basis of the disease than is their numerical impact on the human cancer burden — familial cancers account for only ~ 5% of human cancers, the remainder being 'sporadic' cases. The long sought-after *BRCA1* and *BRCA2* genes that confer strong susceptibility to breast and ovarian cancer account for only

"For unknown reasons, a 'weakness' may occur in specific chromosomes with respect to control of mitosis that at first remains latent and thus is transmitted to a large number of daughter cells … With the beginning of senescence, perhaps this latent weakness becomes manifest in the failure of mitotic control in such a way that when cell division occurs, there is a possibility of generating daughter cells with recurrent genetic abnormalities."

~ 15–20% of the familial cases[53], indicating that most of the genetic risk factors remain to be discovered. Sporadic cancers also have a strong genetic component, particularly for certain tumour types, such as prostate cancer[54]. Why have the genes that are responsible not been found? The reasons are related to the probability that the low-hanging fruit of cancer susceptibility has already been plucked, and that all or most of the familial cases that conform to the 'one gene–one disease' model have already been found. The methods of cancer-susceptibility gene detection that were so successful in the twentieth century might not suffice to find the larger number of low-penetrance genes that control susceptibility to sporadic forms of the disease.

If these genes are hard to find, have weak effects and number in the dozens, if not hundreds, why should we embark on the arduous task of finding them? First, it is clear from animal models of cancer that many low-penetrance genes are extremely powerful preventive agents. For example, skin cancer that is induced by exogenous carcinogens can be almost completely suppressed by the introduction of dominant resistance genes into susceptible mouse strains by breeding with strongly resistant species[55]. Second, identification of human polymorphisms that control sporadic disease susceptibility is one of the 'holy grails' of drug discovery, offering the opportunity for early screening and targets for therapeutic development. Perhaps more importantly, however, gene networks that control the rate-limiting steps of disease progression will provide us with basic insights into cancer biology that are not available by other methods. Although much information will come from large-scale sequencing of human tumour DNA samples, it is clear that this 'tumour-centric' approach will not detect variants in genes that are only expressed in stromal cells, immunocompetent cells or other components of the tumour microenvironment that have important functions as non-cell-autonomous factors in cancer predisposition.

So, what tools are necessary to find these genes? We already know that many genetic modifiers have been mapped — if not actually identified — from mouse models of cancer, and that these alleles engage in genetic interactions that result in the whole effect being greater than the sum of the individual components[56]. The importance of genetic interactions was underlined in evolutionary terms by Sewall Wright, who stated that "a gene is selected on how well its effects fit in with those of the current genetic system"[57]. Wright's proposal was that the driving force of selection *per se* was not necessarily the individual gene variant or mutation, but the combinatorial effect of this variant with the particular constellation of other variants within the host genetic background. He was referring to evolutionary selection, but the same principle applies to selection of tumour cells, in that certain mutations are selected when the genetic background (including occurrence of other mutations) is appropriate. In the development of colon cancer, for example, *TP53* mutations seem to be necessarily preceded by mutations in the adenomatous polyposis coli (APC) gene[58], presumably because *TP53* mutations in normal colon cells do not provide the appropriate selective advantage. Similarly, specific low-penetrance cancer-predisposition genes might only confer susceptibility or resistance to cancer in a particular context: the genetic topography has to be appropriate to observe functional interactions.

Mouse models have been adapted for the study of cancer gene interactions, whereas human-cancer-susceptibility studies have focused on identifying genes with relatively strong effects that are detectable by classical linkage analysis in families or by association studies using candidate gene polymorphisms. The identification of many interacting low-penetrance alleles for human cancer susceptibility might require

insights derived from combinations of mouse models to identify the candidate interacting loci. By analogy with high-penetrance familial cancer genes, such as *RB* and *TP53*, it might be expected that the low-penetrance alleles will influence the genetic pathways adopted by tumours, leaving 'signature patterns' that could ultimately help to identify the crucial polymorphisms. Advances in high-throughput technologies will lead to a complete characterization of all possible somatic genetic alterations at the sequence level in human cancers. This information, together with the identification of the germ-line variants that contribute to cancer susceptibility, will hopefully explain the relationship between inherited predisposition genes and those that acquire mutations during tumour growth and progression. One prediction from mouse genetics is that allele-specific changes in tumours will be an important factor in determining individual cancer risk, but a large-scale study of this question in humans has not yet been attempted.

The characterization of the complex network of interactions that influence cancer development will be facilitated by the emergence of novel microarray-based technologies, such as BAC (bacterial artificial chromosome) microarrays for the high-resolution detection of genetic changes in tumours[59], or cDNA-based, oligonucleotide-based or high-throughput proteomics approaches to detecting changes in gene expression[60,61]. Many laboratories are building computer models of gene networks and, indeed, of whole cell-signalling pathways, in an attempt to simulate the complexities of living systems[62] (also see online links box).

One of the most powerful weapons in the fight against cancer is now within our grasp — the complete sequences of the human and mouse genomes. Our newly acquired ability to call up the array of genes and their sequences has already transformed our approaches to cancer genetics, enabling existing technologies and facilitating the development of spectacular conceptual and practical advances. All of these tools provide a formidable armoury of weapons that will help us to emerge from the twenty-first century with the problems posed by this devastating disease under control. It is unfortunate that we no longer have Boveri to turn to for accurate predictions of where we are headed.

*Allan Balmain is at the Cancer Research Institute, UCSF, San Francisco, California 94143-0875, USA. e-mail: abalmain@cc.ucsf.edu*


1. Mendel, G. Versuche uber pflanzen hybriden. *Verh. Naturforsch. Ver. Brunn.* **4**, 3–47 (1866).
2. Wilson, E. B. *Erinnerungen an Theodor Boveri* (ed. Roentgen, W. C.) (J. C. B. Mohr, Tuebingen, Germany, 1918).
3. Boveri, T. Uber mehrpolige mitosen als mittel zur analyse des zellkerns. *Verh. D. Phys. Med. Ges. Wurzberg N. F.* **35**, 67–90 (1902).
4. Boveri, T. Uber die konstitution der chromatischen kernsubstanz. *Verh. D. Zool. Ges.* **13**, (1903).
5. Boveri, T. Ergebnisse uber die konstitution der chromatischen substanz des zellkerns (Gustav Fischer, Jena, 1904).
6. Boveri, T. In *Zur Frage der Entstehung Maligner Tumoren* 1–64 (Gustav Fisher, Jena, 1914).
7. 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).
8. Bishop, J. M. Enemies within: the genesis of retroviral oncogenes. *Cell* **23**, 5–6 (1981).
9. Nowell, P. & Hungerford, D. Chromosomes of normal and leukemic human leucocytes. *J. Natl Cancer Inst*. **25**, 85 (1960).
10. Rowley, J. D. A new consistent chromosomal abnormality in chronic myelogenous leukemia. *Nature* **243**, 290–293 (1973).
11. Heisterkamp, N. *et al.* Localization of the *c-abl* oncogene adjacent to a translocation break point in chronic myelocytic leukaemia. *Nature* **306**, 239–242 (1983).
12. 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).
13. Nowell, P. C., Rowley, J. D. & Knudson, A. G. Jr. Cancer genetics, cytogenetics — defining the enemy within. *Nature Med.* **10**, 1107–1111 (1998).
14. Shih, C. *et al.* Passage of phenotypes of chemically transformed cells via transfection of DNA and chromatin. *Proc. Natl Acad. Sci. USA* **11**, 5714–5718 (1979).
15. Tabin, C. J. *et al.* Mechanism of activation of a human oncogene. *Nature* **300**, 143–149 (1982).
16. Reddy, E. P., Reynold, R. K., Santos, E. & Barbacid, M. A point mutation is responsible for the acquisition of transforming properties of the *T24* human bladder carcinoma oncogene. *Nature* **300**, 149–152 (1982).
17. Taparowsky, E. *et al.* Activation of the *T24* bladder carcinoma transforming gene is linked to a single amino acid change. *Nature* **300**, 762–765 (1982).
18. Balmain, A. & Pragnell, I. B. Mouse skin carcinomas induced *in vivo* by chemical carcinogens have a transforming Harvey-ras oncogene. *Nature* **303**, 72–74 (1983).
19. Sukumar, S., Notario, V., Martin-Zanca, D. & Barbacid, M. Induction of mammary carcinomas in rats by nitroso-methylurea involves malignant activation of *H-ras-1* locus by single point mutations. *Nature* **306**, 658–661 (1983).
20. Quintanilla, M., Brown, K., Ramsden, M. & Balmain, A. Carcinogen-specific mutation and amplification of the *Ha-ras* gene during mouse skin carcinogenesis. *Nature* **322**, 78–80 (1986).
21. 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).
22. Whyte, P. *et al.* Association between an oncogene and an anti-oncogene: the adenovirus E1A proteins bind to the retinoblastoma gene product. *Nature* **334**, 124–129 (1988).
23. Weinberg, R. A. The retinoblastoma protein and cell cycle control. *Cell* **81**, 323–330 (1995).
24. Knudson, A. G. Jr. Mutation and cancer: statistical study of retinoblastoma. *Proc. Natl Acad. Sci. USA* **68**, 820–823 (1971).
25. Cavenee, W. K. *et al.* Expression of recessive alleles by chromosomal mechanisms in retinoblastoma. *Nature* **305**, 779–781 (1983).
26. Groden, J. *et al.* Identification and characterization of the familial adenomatous polyposis coli gene. *Cell* **66**, 589–600 (1991).
27. Nishisho, I. *et al.* Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. *Science* **253**, 665–669 (1991).
28. Kinzler, K. W. *et al.* Identification of FAP locus genes from chromosome 5q21. *Science* **253**, 661–665 (1991).
29. Futreal, P. A. *et al. BRCA1* mutations in primary breast and ovarian carcinomas. *Science* **266**, 120–122 (1994).
30. Miki, Y. *et al.* A strong candidate for the breast and ovarian cancer susceptibility gene *BRCA1*. *Science* **266**, 66–71 (1994).
31 Lane, D. P. & Crawford, L. V. T antigen is bound to a host protein in SV40-transformed cells. *Nature* **278**, 261–263 (1979).
32. 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).
33. Wolf, D. & Rotter, V. Inactivation of p53 gene expression by an insertion of Moloney murine leukemia virus-like DNA sequences. *Mol. Cell. Biol.* **4**, 1402–1410 (1984).
34. Wolf, D. & Rotter, V. Major deletions in the gene encoding the p53 tumor antigen cause lack of p53 expression in HL-60 cells. *Proc. Natl Acad. Sci. USA* **82**, 790–794 (1985).
35. Mowat, M., Cheng, A., Kimura, N., Bernstein, A. & Benchimol, S. Rearrangements of the cellular p53 gene in erythroleukaemic cells transformed by Friend virus. *Nature* **314**, 633–636 (1985).
36. Fearon, E. R., Hamilton, S. R. & Vogelstein, B. Clonal analysis of human colorectal tumors. *Science* **238**, 193–197 (1987).
37. Baker, S. J. *et al.* Chromosome 17 deletions and p53 gene mutations in colorectal carcinomas. *Science* **244**, 217–221 (1989).
38. Baker, S. J., Markowitz, S., Fearon, E. R., Willson, J. K. & Vogelstein, B. Suppression of human colorectal carcinoma cell growth by wild-type p53. *Science* **249**, 912–915 (1990).
39. Lane, D. P. p53 guardian of the genome. *Nature* **358**, 15–16 (1992).
40. Malkin, D. *et al.* Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas and other neoplasms. *Science* **250**, 1233–1238 (1990).
41. Loeb, L. A., Springgate, C. F. & Battula, N. Errors in DNA replication as a basis of malignant change. *Cancer Res.* **34**, 2311–2321 (1974).
42. Ionov, Y., Peinado, M. A., Malkhosyan, S., Shibata, D. & Perucho, M. Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic neoplasia. *Nature* **363**, 558–561 (1993).
43. Thibodeau, S. N., Bren, G. & Schaid, D. Microsatellite instability in cancer of the proximal colon. *Science* **260**, 816–819 (1993).
44. Parsons, R. *et al.* Hypermutability and mismatch repair deficiency in RER+ tumor cells. *Cell* **75**, 1227–1236 (1993).
45. Evan, G. I. & Vousden, K. H. Proliferation, cell cycle and apoptosis in cancer. *Nature* **411**, 342–348 (2001).
46. Blackburn, E. H. & Challoner, P. B. Identification of a telomeric DNA sequence in *Trypanosoma brucei*. *Cell* **36**, 447–457 (1984).
47. Hastie, N. D. *et al.* Telomere reduction in human colorectal carcinoma and with ageing. *Nature* **346**, 866–869 (1990).
48. Chin, L. *et al.* p53 deficiency rescues the adverse effects of telomere loss and cooperates with telomere dysfunction to accelerate carcinogenesis. *Cell* **97**, 527–538 (1999).
49. Nakamura, T. M. *et al.* Telomerase catalytic subunit homologs from fission yeast and human. *Science* **277**, 955–959 (1997).
50. Meyerson, M. *et al.* hEST2 the putative human telomerase catalytic subunit gene, is up-regulated in tumor cells and during immortalization. *Cell* **90**, 785–795 (1997).
51. Bodnar, A. G. *et al.* Extension of life-span by introduction of telomerase into normal human cells. *Science* **279**, 349–352 (1998).
52. Vaziri, H. & Benchimol, S. Reconstitution of telomerase activity in normal human cells leads to elongation of telomeres and extended replicative life span. *Curr. Biol.* **8**, 279–282 (1998).
53. Ponder, B. Cancer genetics. *Nature* **17**, 336–341 (2001).
54. Lichtenstein, P. *et al.* Environmental and heritable factors in the causation of cancer — analyses of cohorts of twins from Sweden, Denmark, and Finland. *N. Engl. J. Med.* **343**, 78–85 (2000).
55. Balmain, A. & Nagase, H. Cancer resistance genes in mice: models for the study of tumour modifiers. *Trends Genet*. **14**, 139–144 (1998).
56. Fijneman, R. J., de Vries, S. S., Jansen, R. C. & Demant, P. Complex interactions of new quantitative trait loci, Sluc1, Sluc2, Sluc3, and Sluc4, that influence the susceptibility

to lung cancer in the mouse. *Nature Genet*. **14**, 465–467 (1996).
57. Wright, S. Genic and organismic selection. *Evolution* **34**, 825–843 (1980).
58. Kinzler, K. W. & Vogelstein, B. Gatekeepers and caretakers. *Nature* **386**, 761–763 (1997).
59. Albertson, D. G. *et al*. Quantitative mapping of amplicon structure by array CGH identifies *CYP24* as a candidate oncogene. *Nature Genet*. **25**, 144–146 (2001).
60. Perou, C. M. *et al.* Molecular portraits of human breast tumours. *Nature* **406**, 747–752 (2000).
61. Haab, B. B., Dunham, M. J. & Brown, P. O. Protein microarrays for highly parallel detection and quantitation of specific proteins and antibodies in complex solutions. *Genome Biol.* **2**, 1–13 (2001).
62. Gibbs, W. W. Cybernetic cells. *Sci. Am*. **285**, 52–57 (2001).

Acknowledgements
Work in the author's laboratory has been supported mainly by the Cancer Research Campaign (UK) and by the National Cancer Institute (USA). I am grateful to colleagues and the anonymous reviewers for useful comments on the manuscript.

## Online links

**DATABASES**
**The following terms in this article are linked online to:**
**CancerNet:** http://cancernet.nci.nih.gov/index.html
chronic myeloid leukaemia
**LocusLink:** www.ncbi.nlm.nih.gov/LocusLink/
*APC* | *BRCA1* | *BRCA2* | HRAS | *TP53* | *Trp53* | *RB*
**OMIM:** www.ncbi nlm.nih.gov/Omim/
adenomatous polyposis coli | hereditary non-polyposis colorectal cancer | xeroderma pigmentosum

**FURTHER INFORMATION**
**Boveri information web sites:**
www.biozentrum.uni-wuerzburg.de/about/boveri.html;
http://zygote.swarthmore.edu/fert6b.html
**Computer models of cellular signalling:**
www.cellularsignaling.org
**Human Genome Sequence:** http://www.ncbi.nlm.nih.gov/
**Mendel's Genetics:**
http://anthro.palomar.edu/mendel/mendel_1.htm
**MendelWeb:** www.netspace.org/MendelWeb/

TIMELINE

# Tobacco and the global lung cancer epidemic

*Robert N. Proctor*

Tobacco is the world's single most avoidable cause of death. The World Health Organization has calculated that the 5.6 trillion cigarettes smoked per year at the close of the twentieth century will cause nearly 10 million fatalities per year by 2030. Lung cancer is the most common tobacco-related cause of cancer mortality, with one case being produced for every 3 million cigarettes smoked. How was the global lung cancer epidemic recognized, and what can we expect in the future?

The tobacco plant is native to the Americas; archaeological evidence indicates that Mayans were smoking the leaf as early as the first century BC (FIG. 1). Columbus discovered the Arawaks using dried tobacco leaves in several curious rituals, and was offered the plant as a gift. Several of his men took up smoking, and the habit was soon exported to Europe and the rest of the world. Tobacco was used sporadically throughout the seventeenth and eighteenth centuries, although objections were sufficiently strong in many places to have bans enacted. A Chinese imperial edict of 1612 barred growing or smoking the leaf, and the city of Berlin banned smoking in 1723[1]. Smoking was illegal in 14 American states as late as 1921, although none of these bans would survive the decade.

Tobacco has been used in many different forms. Native Americans 'drank' the smoke in hand-rolled palm or maize leaves, whereas European sailors tended to prefer chewing to avoid the hazards of fire. Cigarettes were not popular until the nineteenth century; the French Revolution gave snuff an aristocratic odour and cleared a path for 'little cigars'[2]. Health effects were limited in these early years, however, as the methods most commonly used to cure the leaf made the smoke too harsh to inhale. Cigarettes were also time-consuming to manufacture: the women and girls who hand-rolled cigarettes in the mid 1800s could usually roll only about 200 per day.

Cigarette production was given an enormous boost in 1880 with the invention of the Bonsack cigarette-rolling machine (FIG. 2), which could churn out more than 100,000 cigarettes per day. W. Duke, Sons and Company of Durham, North Carolina, installed two such machines in 1884, allowing them to produce an unprecedented 744 million cigarettes in a single year. When combined with mass marketing and the invention of safety matches (in 1855), cigarettes quickly became a popular consumer item. Americans smoked only about eight cigarettes per person per year in the 1880s; by the end of the century, this figure would more than quadruple. Cigarettes were included with the rations of soldiers in the First World War, and many of the young men who entered the war as abstainers returned home as addicts. Consumption was further increased by new methods of advertising and government encouragement, following the recognition that tobacco could supply an impressive streak of tax revenues. Tobacco taxes in the United States, for example, went from about $13 million in 1910 to nearly $5 *billion* ($10^9$) some 60 years later. Tobacco provided 8% of Germany's entire national tax income in the 1930s , and China today earns an even higher percentage (~ 10%). Dependence on tax revenues is one of the main reasons why governments have been reluctant to challenge the tobacco juggernaut. One tobacco company

Figure 1 | **The oldest existing illustration of a smoker — a Mayan god.** (Image courtesy of Imperial Tobacco.)