modality. In order to demonstrate an improvement in these subgroups multicentre studies will be required. The improvement in survival produced by even small percentage increments are worthwhile. For example, a 15% improvement in local control rates for carcinoma of the cervix and bladder only could result in increased survival of over 1500 patients annually. The change, however, from an approach based on broad prognostic categories with one therapeutic manoeuvre to that based on multiple subgroups selected for different therapeutic modalities is a considerable change from current practice.

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How are Cancer Associated Genes Activated or Inactivated?

Leanne M. Wiedemann and Gareth J. Morgan

Altered behaviour or the transformation of a cell can result from the abnormal expression of some oncogene products. Elevated or inappropriate expression can result from (i) mutations in the regulatory region of the gene, (ii) aberrant expression of a transcription factor involved in the regulation of the gene, (iii) gene amplification, or (iv) the insertion of a viral promoter upstream of the gene. In addition, an alteration in the product of a proto-oncogene can lead to the acquisition of a transforming activity. Such changes have been shown to include (i) point mutation, (ii) deletion, and (iii) the formation of fusion genes. Finally, the loss of activity of a gene product can contribute to transformation. This can come about by (i) small or large deletions, (ii) point mutations which abolish function or expression of an intact protein, or (iii) mutations which lead to a protein with an activity which can inhibit the suppressor activity of the normal allele.
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INTRODUCTION

MECHANISMS LEADING to the activation or inactivation of genes found to contribute to the formation of a tumour are many and varied. These mechanisms are not necessarily mutually exclusive and several may be involved in the activation or inactivation of a gene product. It is also important to remember that the activation (or inactivation) of a single copy of an individual gene along a signalling pathway is not sufficient to result in a fully malignant change in the behaviour of the cell. In most forms of cancer only one or two of the five or more oncogenic steps have been pin-pointed [1].

This short review is not a complete list of changes known to occur in cancer, but gives a few examples of those that have been characterised to date.

POINT MUTATION

RAS mutations

One of the first oncogenes to be shown to be activated by a point mutation was the human homologue of the Ha-*RAS* gene. The altered activity of the RAS proteins was first identified in a transformation assay where both the appearance and behaviour of an indicator fibroblast cell line, NIH 3T3, was altered following transfection with DNA. The DNA for these experiments originated from the EJ bladder carcinoma cell line. The transforming allele of the *RAS* gene was shown to contain a single amino acid change which had converted the cellular proto-oncogene into an oncogene. Additional activating point mutations have been identified in Ha-*RAS* and other members of the *RAS* gene family [2]. Normal RAS proteins have been shown to bind and hydrolyse GTP to GDP. Many of the mutations have been shown to affect the ability of these proteins to hydrolyse the GTP molecule; since *RAS* transmits a signal when in the GTP-bound state, these mutations result in the constitutive activation of the complex. The molecular consequences of this change are still the subject of intense investigation in a number of laboratories.

Other genes encoding GTP-binding proteins have been identified which have been implicated in tumorogenicity (guilt by association) due to the identification of point mutations in some endocrine tumours. Examples include the *GIP* gene in carcinoma of ovary and adrenal gland and the *GSP* gene in adenoma of the pituitary gland and carcinoma of the thyroid gland [3].

Other activating mutations

In addition, a number of other proto-oncogenes have been shown to acquire point mutations in certain forms of cancer [4]. Point mutations have been identified in c-*FMS* (CSF-1 receptor) in monocytic leukaemias [5] which may result in the constitutive activation of the protein tyrosine kinase activity of the receptor in the absence of the growth factor ligand. Other mutations and structural alterations which can lead to the activation of growth factor receptors are reviewed by Aaronson and Tronick [6].

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INACTIVATION OF TUMOUR SUPPRESSOR GENES

In addition to the activation of genes, point mutations may also result in the inactivation of genes. This may lead to the elimination of a gene product which is necessary for the control of cell proliferation. Gene products of this type have been termed suppressor genes as they may act to suppress a tumour phenotype [7]. One of the first suppressor genes to be identified was the gene, *RB*, implicated in familial and sporadic forms of retinoblastoma [8]. It has been shown that mutations and deletions resulting in loss-of-function or loss of expression of both alleles of the *RB* gene, are necessary for the development of the tumour.

p53 mutations

A large number of point mutations have been identified in the *p53* gene (another tumour-suppressor gene) in colon carcinoma [9] and other solid tumours [10]. Some of the characterised mutations lead to a loss of expression of the protein product, but at least a proportion of the mutations in *p53* are expressed as a protein and may act by binding to and thereby inactivating the normal *p53* protein which is expressed from the other allele [11]. These have been termed dominant-suppressor mutations.

Other targets for mutation

More recently, the genes *MCC* (*m*utated in *c*olorectal *c*arcinoma; [12]) and *DCC* (*d*eleted in *c*olorectal *c*arcinoma; [13]) have been implicated as being targets for mutation. It is known that several genetic changes are required during the development of colorectal carcinoma. During progression from benign to more malignant tumour formation, point mutations or deletions may result in the inactivation of these genes whose normal function may be to suppress tumours of the large bowel.

CHROMOSOMAL TRANSLOCATIONS ACTIVATING PROTO-ONCOGENES

The cytogenetic analysis of human leukaemia has shown characteristic chromosomal abnormalities. These include numerical changes in modal chromosome number, chromosomal deletions and chromosomal rearrangements. The specificity of some of these changes and their association with defined subsets of leukaemia has led to the suggestion that these changes are involved in the causation of leukaemia. The molecular characterisation of translocations has been successful in identifying previously unknown genes, many of which have been shown to be important in either cell growth or differentiation (Table 1).

Many of the consistent translocations in the lymphoid malignancies have been at least partially characterised at the molecular level largely because of the availability of probes to the immunoglobulin (Ig) and T cell receptor (*TCR*) genes [14]. The Ig heavy chain gene at 14q32, the Ig κ light chain gene at 2p12 or the Ig λ light chain gene at 22q11 are common sites of translocation in B lineage leukaemias. Similarly, many of the consistent chromosomal translocations in T cell malignancies involve regions where the *TCR* α (14q11), β (7q35), or δ (14q11) genes map. The molecular characterisation of translocations in the myeloid leukaemias has been slower, because of the need to generate suitable probes for the chromosomal regions of interest.

TAL1 activation in T-cell leukaemia

The identification of genes involved in translocations can sometimes lead to the discovery of deletions or mutations which are not cytogenetically visible. A putative transcription factor encoding gene, *TAL1*, has been identified [15] at the 1p32 breakpoint in the t(1;14)(p32;q11). This translocation is only observed in 7% of the cases of T-ALL, but when other T-cell leukaemias were screened with a *TAL1* probe, 30% were found to have a specific 90 kb intrachromosomal deletion in the 5′ end of the gene [16]. As yet, the molecular consequences of this deletion are unclear.

Table 1. Some molecularly characterised chromosomal translocations

T Lymphoid		
t(7;19)(q34;p13)	T-ALL	TCR β, LYL1
t(1;14)(p32;q11)	T-ALL	TAL1, TCR δ
t(11;14)(p15;q11)	T-ALL	TTG1, TCR δ
t(11;14)(p15;q11)	T-ALL	RHOM-1, TCR δ
B Lymphoid		
t(1;19)(q23;p13)	pre B-cell ALL	PBX, E2A
t(8;14)(q24;q32)	Burkitts lymphoma	MYC, IgH locus
t(2;8)(p12;q24)	Burkitts lymphoma	κ light chain, MYC
t(8;22)(q24;q11)	Burkitts lymphoma	MYC, λ light chain
t(11;14)(q13;q32)	CLL	bcl1, IgH locus
t(14;18)(q32;q21)	Follicular lymphoma	IgH locus, BCL2
Myeloid		
t(9;22)(q34;q11)	CML	ABL, BCR
t(6;9)(p23;q34)	AML	DEK, CAN
t(15;17)(q22;q11)	AML M3	MYL, RARα

The deregulation of gene expression

Chromosomal translocations in Burkitts lymphoma. One of the first chromosomal translocations to be characterised at the molecular level was the t(8;14)(q24;q32) chromosomal translocation commonly seen in Burkitts lymphoma and B cell ALL [17]. Variant translocations have also been described, t(2;8)(p12;q24) and t(8;22)(q24;q11). All three involve the c-*myc* proto-oncogene located at 8q24 which recombines with the Ig heavy chain (14q32), Ig κ light chain (2p12) or Ig λ light chain (22q11) gene.

The c-*myc* gene product is known to bind to DNA [18] and is thought to play a role in maintaining cells in cycle. The close proximity of c-*myc* to the enhancer region of the Ig locus can result in the inappropriate expression of MYC protein. Further mutations in the 5′ region may also affect transcriptional regulation [19]. Variant translocations into regions 3′ of the c-*myc* coding sequence may result in increased efficiency of translation or stability of the mRNA product [20].

The t(14;18) and follicular lymphoma

In the t(14;18) found in 90% of follicular lymphoma, the expression of a gene *BCL-2* [21] is deregulated due to its new chromosomal location in close proximity to the IgH enhancer. The expression of this product in lymphoid cells prevents their response to factors which bring about apoptosis (programmed cell death). This may result in an expanded pool of target progenitor cells at risk of acquiring secondary genetic mutations and progression to the clinical phenotype of follicular lymphoma [22].

The formation of hybrid genes

Philadelphia translocation. A balanced chromosomal translocation can also result in the formation of a hybrid gene, with either an enhanced or novel function. Chronic myeloid leu-

kaemia (CML) is consistently associated with the fusion of *BCR* (chromosome 22q11) and *ABL* genes (chromosome 9q34) [23, 24]. This leads to the generation of a hybrid p210 BCR/ABL protein with activated protein kinase activity [25]. An alternative form of this translocation is frequently seen in Ph-positive acute leukaemia; in this case a more 5′ portion of BCR is directly joined to ABL resulting in a novel p190 product [26, 27].

The junctions between chromosome 22 and chromosome 9 occur within the introns of the *BCR* and *ABL* genes such that the spliced mRNA product of a Ph-chromosome can produce only a limited number of exon/exon junctions [28] despite the large variation in location of the DNA breakpoints. The substrate and mechanism of action of this protein tyrosine kinase is still to be elucidated.

The t(15;17) *and promyelocytic leukaemia.* The t(15;17) is found in almost all cases of AML M3. As a result of the translocation the retinoic acid receptor α gene (*RARα*), located at 17q22, is disrupted. A hybrid gene is formed between a gene called *MYL* and *RARα* [29]. The region of the *RARα* which *MYL* replaces is thought to determine the DNA target specificity of the receptor. The hybrid molecule does not function normally in the presence of the *RARα* ligand, retinoic acid. As a result, differentiation appears to be blocked at the level of the promyelocyte in the cell harbouring the translocation. The block can be overcome at least for a time, with large doses of all-trans-retinoic acid [30]. This implies that the hybrid affects, in the cell harbouring the translocation, the ability of the gene product encoded by the normal allele to respond to retinoic acid in a dominant manner; high doses of retinoic acid are able to overcome this either directly or by alternative routes.

The t(1;19) *and pre B-ALL.* In pre B-cell ALL with a t(1;19)(q23;p13), involvement of a putative DNA binding transcription factor has been noted. The *E2A* and *PBX* genes which have been identified at the breakpoints of this translocation show homology with gene transcription factors [31]. The chimaeric gene consists of the DNA binding domain of PBX fused to the transcriptional activating domain of E2A [32]. This probably results in the transcriptional activation of genes not normally expressed in the B-cell progenitor carrying the translocation.

THE EXPLOITATION OF MOLECULAR ALTERATIONS

The elucidation of the genetic damage which underlie tumour growth, in addition to helping to explain the mechanisms leading to cancer, also allows the application of a variety of molecular techniques to the diagnosis and management of cancer patients. Techniques including DNA restriction enzyme analysis, the polymerase chain reaction, *in situ* hybridisation, and antigen-detection with antibodies have been shown to be of use in the characterisation and monitoring of various forms of cancer.

Clinical studies have shown that the gene, *erb*B-2 (also called *NEU*) which encodes a growth factor receptor, is frequently over-expressed in carcinoma of the breast and may be a prognostic indicator of poor survival [33, 34]. Amplification of another proto-oncogene, N-*myc*, has been observed in neuroblastomas and shown to correlate with poor prognosis [35]. The ability to detect residual tumour cells by virtue of their unique molecular alterations, may eventually be used to determine whether the intensity or duration of chemotherapeutic treatment should be altered [36].

The obvious hope is that the increased understanding of the events leading to cancer will provide targets for therapy. The revelation that the RARα is altered in acute promyelocytic leukaemia (see above) helps to explain the efficacy of treatment protocols with retinoic acid. The identification of tumour specific products may allow the production of immunological or pharmaceutical reagents designed to inhibit the dominant function of an activated oncogene, such as the BCR/ABL protein tyrosine kinase observed in Ph-positive leukaemia. Alternatively, replacement of the product of a deleted suppressor gene may become a possibility.

1. Hunter T. Cooperation between oncogenes. *Cell* 1991, **64**, 249–270.
2. Bos JL. The *ras* family and human carcinogenesis. *Mutat Res* 1988, **195**, 244–271.
3. Lyons J, Landis CA, Harsh G, *et al.* Two G protein oncogenes in human endocrine tumors. *Science* 1991, **249**, 655–659.
4. Bishop JM. Molecular themes in oncogenesis. *Cell* 1991, **64**, 235–248.
5. Ridge SA, Worwood M, Oscier D, Jacobs A, Padua RA. FMS mutations in myelodysplastic, leukemic, and normal subjects. *Proc Natl Acad Sci USA* 1990, **87**, 1377–1380.
6. Aaronson SA, Tronic SR. Growth factor signalling pathways and their alterations in human tumors. *Forum: Trends Exp Clin Med* 1991, **1**, 14–29.
7. Marshall CJ. Tumour suppressor genes. *Cell* 1991, **64**, 313–326.
8. 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, **232**, 643–646.
9. Baker SJ, Markowitz S, Fearon ER, Willson JKV, Vogelstein B. Suppression of human colorectal carcinoma cell growth by wild-type p53. *Science* 1990, **249**, 912–915.
10. NigroJM, Baker SJ, Preisinger AC, *et al.* Mutations in the p53 gene occur in diverse human tumour types. *Nature* 1989, **342**, 705–708.
11. Levine AJ, Momand J, Finlay CA. The p53 tumour suppressor gene. *Nature* 1991, **351**, 453–456.
12. Kinzler KW, Nilbert MF, Vogelstein B, *et al.* Identification of a gene located at chromosome 5q21 that is mutated in colorectal cancers. *Science* 1991, **251**, 1366–1370.
13. Fearon ER, Cho KR, Nigro JM, *et al.* Identification of a chromosome 18q gene that is altered in colorectal cancers. *Science* 1990, **247**, 49–56.
14. Boehm T and Rabbitts TH. A chromosomal basis of lymphoid malignancy in man. *Eur J Biochem* 1989, **185**, 1–17.
15. Chen Q, Cheng JT, Tasi LH, *et al.* The *TAL* gene undergoes chromosome translocation in T cell leukemia and potentially encodes a helix-loop-helix protein. *EMBO J* 1990, **9**, 415–424.
16. Brown L, Cheng JT, Chen Q, *et al.* Site specific recombination of the TAL-1 gene is a common occurrence in human T-cell leukaemia. *EMBO J* 1990, **9**, 3343–3351.
17. Leder P, Battey J, Lenoir G, *et al.* Translocations among antibody genes in human cancer. *Science* 1983, **222**, 765–770.
18. Luscher B, Eisenman RN. New Light on MYC and MYB. Part 1. *Genes and Development* 1990, **4**, 2025–2035.
19. Showe LC, Croce CM. Chromosome translocation in B and T cell neoplasias. *Seminars in Haematol* 1986, **9**, 237–244.
20. Cory S. Activation of cellular oncogenes in haemopoietic cells by chromosome translocation. *Adv Canc Res* 1986, **47**, 189–234.
21. Cleary ML, Smith SD, Sklar J. Cloning and structural analysis of cDNA for BCL-2 and a hybrid BCL-2/Ig transcript resulting from the t(14;18) translocation. *Cell* 1986, **47**, 19–28.
22. Sawyers CL, Denny CT, Witte ON, Leukemia and the disruption of normal haemopoiesis. *Cell* 1991, **64**, 337–350.
23. Kurzrock R, Gutterman JU, Talpaz M. The molecular genetics of Philadelphia chromosome-positive leukemias. *N Engl J Med* 1988, **319**, 990–998.
24. Wiedemann LM, Karhi KK, Shivji MKK, *et al.* The correlation of bcr rearrangement and p210 *phl/abl* expression with morphological analysis of Ph-negative CML and other myeloproliferative diseases. *Blood* 1988, **71**, 349–355.
25. Ben-Neriah Y, Daley GQ, Mes-Masson AM, Witte ON, Baltimore D. The chronic myelogenous leukemia-specific p210 protein is the product of the *bcr-abl* hybrid gene. *Science* 1986, **233**, 212–214.
26. Chan LC, Karhi KK, Rayter SI, *et al.* A novel abl protein is

expressed in Philadelphia chromosome positive acute lymphoblastic leukaemia. *Nature* 1987, **325**, 635–637.
27. Hermans A, Heisterkamp N, von Lindern M, *et al.* Unique fusion of *bcr* and *c-abl* genes in Philadelphia chromosome positive acute lymphoblastic leukemia. *Cell* 1986, **51**, 33–40.
28. Hermans A, Gow J, Selleri L, *et al.* *bcr-abl* oncogene activation in Philadelphia chromosome-positive acute lymphoblastic leukemia. *Leukemia* 1988, **2**, 628–633.
29. de The H, Chomienne C, Lanotte M, Degos L, Dejean A. The t(15;17) translocation of acute promyelocytic leukaemia fuses the retinoic acid receptor α gene to a novel transcribed locus. *Nature* 1990, **347**, 558–561.
30. Meng-er H, Yu-chen Y, Shu-rong C, *et al.* Use of all-trans retinoic acid in the treatment of acute promyelocytic leukemia. *Blood* 1988, **72**, 5567–572.
31. Nourse J, Mellentin JD,Galili N, *et al.* Chromosomal translocation t(1;19) results in synthesis of a homeobox fusion mRNA that codes for a potential chimeric transcription factor. *Cell* 1990, **60**, 535–545.
32. Hunger SP, Galili N, Carroll AJ, Crist WM, Link MP, Cleary ML. The t(1;19)(q23;p13) results in consistent fusion of E2A and PBX1 coding sequences in acute lymphoblastic leukemias. *Blood* 1991, **77**, 687–693.
33. Slamon DJ, Gololphin W, Jones LA, *et al.* Studies of the HER-2/*neu* proto-oncogene in human breast and ovarian cancer. *Science* 1989, **244**, 707–712.
34. Paterson MC, Dietrich KD, Danyluk J, *et al.* Correlation between c-*erB*-2 amplification and risk of recurrent disease in node-negative breast cancer. *Cancer Res* 1991, **51**, 556–567.
35. Bourhis J, De Vathaire F, Wilson GD, *et al.* Combined analysis of DNA ploidy index and N-*myc* genomic content in neuroblastoma. *Cancer Res* 1991, **51**, 33–36.
36. Lee MS, Chang KS, Cabanillas F, Freireich EJ, Trujillo JM, Stass SA. Detection of minimal residual cells carrying the t(14;18) by DNA sequence amplification. *Science* 1987, **237**, 175–179.

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What are Cancer Genes, and how do they Upset Cell Behaviour?

J.R. Yarnold

Some of the cellular changes underlying the presentation of cancer in a patient can already be understood in terms of mutations affecting specific gene functions. So far, only a few of the mutated genes responsible for carcinogenesis have been identified and these are chiefly involved in deregulation of cell growth rather than with the processes of invasion and metastasis. Proto-oncogenes are important cellular genes which can acquire gain in function mutations as random events in somatic cells. In their mutated, activated forms they are called cellular oncogenes or c-oncs. This distinguishes them from homologous DNA sequences captured by viruses from host cells in the course of retroviral evolution that cause cancers in animal hosts (viral oncogenes or v-oncs). In recent years, loss of function mutations have been identified in regulatory genes that normally serve to constrain cell growth. These are called tumour suppressor genes. Loss of function mutations may be transmitted in the germline, as in hereditary retinoblastoma, or arise *de novo* in somatic cells. The normal molecular mechanisms disrupted by mutations in tumour suppressor genes include processes regulating progression through the cell cycle.
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SOMATIC MUTATION THEORY AND MULTISTAGE CARCINOGENESIS

SOME OF the cellular changes underlying tumour growth, invasiveness and metastatis can already be understood in terms of altered function in a tiny percentage of the several tens of thousands proteins coded in the human genome. Altered gene function arises from altered DNA sequences (mutations) which arise either spontaneously, e.g. from uncorrected errors in DNA replication, or from exposure to exogenous carcinogens. For example, the benzopyrene content of cigarette smoke is probably responsible for point mutations that alter the p53 gene sequence and activity in many lung cancers [1]. Mutational events in critical genes presumably confer some kind of survival or growth advantage on the affected cells, so that descendants accumulate genetic damage over successive generations [2, 3].

Progressive alterations in cell morphology and tissue organisation can be observed for several years before the appearance of invasive disease, e.g. epithelial hyperplasia, epithelial dysplasia and carcinoma *in situ*. The molecular mechanisms underlying these pathological changes are best described in colon cancer where the accumulation of mutations in several genes correlates with progression via epithelial dysplasia, adenoma and carcinoma *in situ* to frank malignancy [4–6]. Colon cancer offers the best illustration so far of multiple somatic mutations underlying multistage carcinogenesis. As we shall see later, the inheritance of mutations in the germ-line also contribute in some individuals with cancer.

Mutational (genetic) events in cancer cells are not the only factors affecting cancer development; its just that they are easier to identify than environmental (epigenetic) influences that also affect the evolution of malignancy. As evidence of this, the

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