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Tumour suppressor genes

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Summary

Genes that can inhibit the expression of the tumorigenic phenotype have been detected by the fusion of normal and malignant cells, the phenotypic reversion of *in vitro* transformants, the induction of terminal differentiation of malignant cell lineages, the loss of 'recessive cancer genes', the discovery of regulatory sequences in the immediate vicinity of certain oncogenes, and the inhibition of tumour growth by normal cell products. Such tumour suppressor genes will probably turn out to be as, if not more, diversified as the oncogenes. Consideration of both kinds of genes may reveal common or interrelated functional properties.

Introduction

The category of genes that can suppress transformation or tumorigenicity may be as diversified as, or even more, diversified than the oncogenes. The constitutive activation of a 'growth-factor oncogene' for example, may be cancelled by the loss or dysfunction of the corresponding receptor, by a roadblock elsewhere within the complex pathway of signal transmission, and by changes in the responding target. Oncogene-induced blocks to cell maturation may be overcome by strong inducers or circumvented by the use of alternative pathways. In this article I will review the fragmentary but firm evidence that shows the existence of such mechanisms.

Tumour-suppressing genes have been detected in the following systems: (i) Fusion of normal and malignant cells leads to the suppression of the tumorigenic phenotype in the majority of the combinations where the hybrid maintains a relatively complete chromosome complement. Reappearance of tumorigenicity is accompanied by chromosome losses. The loss of certain normal parent-derived chromosomes appears to be particularly important (Harris *et al.* 1969; Harris, 1971; Klein *et al.* 1971; Wiener *et al.* 1974; Stanbridge, 1987; Klinger, 1982; Klinger & Shows, 1983; Sager, 1985). (ii) Morphological and nontumorigenic revertants have been isolated from both virally and chemically induced transformants (Bassin & Noda, 1987; Sachs, 1987). They are not necessarily generated by the loss or down-regulation of the original transforming gene. (iii) Differentiation blocks can be bypassed by the temporary down-regulation of temperature-sensitive oncogenes or by exposure to strong differentiation-inducing signals. (iv) Loss or mutational inactivation of 'recessive cancer genes' plays an essential role in the genesis of retinoblastoma, Wilms' tumour, and osteosarcoma, indicating that the normal alleles of these genes can counteract neoplastic transformation in the corresponding tissues (Knudson, 1987; Benedict, 1987). (v) Regulatory sequences capable of preventing

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illegitimate activation (Vande Woude *et al.* 1987; Verma, 1986) have been identified in the immediate vicinity of certain oncogenes (for example, c-*mos* and c-*fos*). (vi) Tumour growth can be inhibited by diffusible products released by surrounding normal cells.

Suppression of tumorigenicity by somatic hybridization

A large variety of spontaneous, virally, and chemically induced tumours become low- or nontumorigenic after fusion with fibroblasts, lymphocytes, or macrophages (Harris *et al.* 1969; Harris, 1971; Klein *et al.* 1971; Wiener *et al.* 1974; Stanbridge, 1987; Klinger, 1982; Sager, 1985; Bassin & Noda, 1987). Reappearance of tumorigenicity after chromosome loss was found to occur at variable rates, depending on the stability of each hybrid combination.

The suppression of tumorigenicity by cell hybridization can be discussed in genetic or epigenetic terms that are not mutually exclusive. If genetic losses play an essential role in the evolution of the malignant phenotype, the normal cell genome may act by genetic complementation. In cases where the neoplastic transformation is due to a blockage of maturation, for example, by a dominantly acting oncogene, the normal partner cell may impose its own differentiation program on the hybrid. Stanbridge has concluded that 'the hybrid cell takes on the phenotypic signature of the normal parental cell, regardless of the origin of the malignant parental cell' (Stanbridge, 1987).

The identification of chromosome pairs of the normal parent that are regularly lost from the high malignant segregants was helpful to map the location of the relevant suppressor genes. Human intraspecies hybrids were studied most extensively. Stanbridge *et al.* (1981) found that the reexpression of tumorigenicity in hybrids of HeLa cells and normal fibroblasts was associated with the loss of one copy of chr 11 and one copy of chr 14. Klinger's group (Kaelbling & Klinger, 1986; Klinger & Kaelbling, 1986; Kaelbling *et al.* 1986) provided similar evidence for human chr 11, whereas Benedict *et al.* (1984) implicated human chr 1 and possibly chr 4 in the suppression of the HT 1080 fibrosarcoma by normal fibroblasts. This is not necessarily a contradiction. The tumorigenic phenotype may be suppressed by functionally different mechanisms, depending on the transforming gene and the phenotype of the normal partner cell. The two malignant partners of these crosses, HeLa and HT 1080, produced nontumorigenic hybrids when fused with each other (Weissman & Stanbridge, 1983), suggesting genetic complementation between cells that carry different genetic lesions. HT 1080 carries a mutationally activated N-*ras* allele (Benedict *et al.* 1984). Corresponding losses were found in tumours that carry mutated *ras*, including chemically induced mouse skin carcinomas (Quintanilla *et al.* 1986), thymic lymphomas (Guerrero *et al.* 1985) and a variety of human tumours and derived cell lines (Taparowsky *et al.* 1982; Santos *et al.* 1984; Kraus *et al.* 1984; Fearon *et al.* 1985). It is therefore conceivable that the normal *ras* may antagonize the tumorigenic effect of the mutated allele. It was particularly suggestive that the progression of chemically induced mouse skin papillomas to carcinoma was

accompanied by the amplification of the mutated *ras* or the loss of the normal allele or both (Quintanilla *et al.* 1986).

The suppression of tumorigenicity in hybrids between normal cells and tumour cells transformed by activated oncogenes may occur at different levels. Down-regulation of transcription has been demonstrated for v-*src* (Dyson *et al.* 1982; Wyke & Green, 1986), but it is more the exception than the rule. It is more frequent that suppression acts beyond the level of oncoprotein expression. This was found in the SV40 system (Sager, 1986; Howell, 1982) and particularly often in relation to *ras*-transformed cells.

Geiser *et al.* (1986) fused the human EJ bladder carcinoma line, which carries a transforming, mutation-activated *ras* gene, with normal fibroblasts. The hybrids retained the transformed phenotype *in vitro*, but did not grow in nude mice. Tumorigenic segregants appeared on serial cultivation. The mutated *ras* p21 protein was present at the same level in tumorigenic and nontumorigenic hybrids. Transfection with c-H-*ras*-expressing constructs increased the amount of p21, but did not induce tumorigenicity. Suppression of transformation in the absence of any change in p21 expression was also demonstrated in a Chinese hamster (Craig & Sager, 1985) and a mouse system (Rabinowitz & Sachs, 1970; Greenberger *et al.* 1976). In the latter study, flat revertants isolated from Kirsten sarcoma virus-transformed murine fibroblasts still contained a functionally intact viral oncogene, as shown by rescue experiments. Their p21 level was as high as in the original transformants, but they were resistant to retransformation by activated *ras* of either cellular or viral origin. Somatic hybridization of the revertants with both nontransformed and transformed cells of the same lineage generated nontransformed hybrids. The revertants could also suppress *src*, *fes*, K-, H-, and N-*ras* and mutated human H-*ras* transformants, but not *mos*, *sis*, *fms*, *ras*, polyoma, SV40, and chemically transformed cells of the same origin.

Src and *fes* encode oncoproteins unrelated to *ras*. The common suppression pattern suggests that the dominant reversion imposes a block on a transformation pathway that converges in these three transformants. *raf* and *mos* are believed to act at a level beyond the *ras*-dependent signalling pathway (Rapp *et al.* 1987). The analysis of the suppression patterns provides a new approach towards the definition of these pathways in cells transformed by different oncogenes. The mapping of suppressor genes by the relatively cumbersome method of somatic hybridization will probably be replaced by the more direct microcell-mediated transfer of single chromosomes, as exemplified by the recent report of Weissman *et al.* (1987) on the suppression of Wilms' tumour cells by fusion with a minicell containing chromosome 11.

Reversion

The isolation of nontransformed and/or nontumorigenic variants from cultures of transformed cells has provided another source of information on tumour suppressor genes. It is easy to see that many different types of revertants must exist. Each

regulatory or structural change that pushes the cell forward along the pathway of progression must have a counterpart that may cause reversion. Reversion can only be detected at the population level if the growth of the original malignant cell is inhibited, however. This requires special techniques. Bassin & Noda (1987) have subdivided revertants into an oncoprotein-related and a target-related category. The former arise by the loss or inactivation of a transforming gene, whereas the latter continue to express the transforming protein, but are phenotypically normal or quasi-normal.

Revertants with a defective oncoprotein are relatively trivial. They usually arise in cultures of virally transformed cells and are susceptible to retransformation by the same agent. Target-related revertants are resistant to retransformation. Noda *et al.* (1983) increased the probability of isolating such revertants by starting with doubly infected cells that carried two copies of the viral v-Ki *ras* gene. *N'*-methyl-*N*-nitrosoguanidine (MNNG)-mutagenized cultures contained approximately 10^{-7} revertants that were more flattened, cloned less well in agarose, lacked tumorigenicity, and had an increased chromosome number. They contained the same two v-Ki *ras* copies as the transformant, grew equally well in low serum and produced the same high amounts of the p21 *ras* protein and transforming growth factor-alpha (TGF-alpha). It was suggested that they had arisen by a change in the transformation pathway, occurring at some point beyond the interaction of TGF with its receptor. A possible clue about the nature of this change was provided by the finding that the characteristically reduced tropomyosin content of the *ras*-transformed cells was restored to the control level in the revertants (Cooper *et al.* 1985).

Another study by the same group (Noda *et al.* 1985) has shown that the same gene can act in a transforming or a suppressing capacity, depending on the target cell. Activated *ras* and v-*src* genes can transform fibroblasts, but suppress growth of PC 12 cells, which were derived from rat pheochromocytoma. PC 12 cells can multiply indefinitely in growth medium, but differentiate into sympathetic neurones after exposure to nerve growth factor. The two viral oncogenes mimic the activities of NGF. It was suggested that they may induce the same intracellular signals in both kinds of cells, but elicit different responses, depending on the properties of the target cell.

Recessive cancer genes – a special category of tumour suppressor genes?

Molecular analysis has fully confirmed the ingenious theory of Knudson that retinoblastoma arises by the loss of both alleles at the same locus (RB-1). The gene is localized at 13q14 on the human chromosome map (for review see Knudson, 1987). In familial retinoblastoma, a defective RB-1 allele is transmitted through the germline. It may be associated with a deletion at 13q14.2, but is more frequently invisible at the cytogenetic level. The second change occurs during somatic development. It may arise by the loss of one chr 13 with or without the duplication of

the other, or, less frequently, by somatic crossing over or by interstitial deletion (Cavenee *et al.* 1983).

Recently, a cDNA fragment that corresponds to a gene that spans over at least 70 kb of human chr 13q14 has been cloned. The gene was sequenced and identified as the retinoblastoma susceptibility gene (Friend *et al.* 1986; Lee *et al.* 1987; Fung *et al.* 1987). It is expressed in many tumour cells and also in foetal retina, but not at all, or only in a truncated form, in retinoblastomas and osteosarcomas. It remains to be shown whether this gene is capable of reverting some of the malignant properties of retinoblastoma and osteosarcoma, when introduced in an appropriately active form.

A gene localized at 11p13 appears to play a similar role in Wilms' tumour (Francke *et al.* 1979; Ladda *et al.* 1974; Riccardi *et al.* 1980) and perhaps in hepatoblastoma and embryonal rhabdomyosarcoma as well (Koufos *et al.* 1985). Similar genetic losses may be involved in some solid tumours in adults. The 3p14 region is frequently deleted in renal carcinoma and in small cell carcinoma of the lung (Wang & Perkins, 1984; Pathak *et al.* 1982; Kovacs *et al.* 1987; Kovacs *et al.* 1988; Heim & Mitelman, 1987; Yoshida *et al.* 1986; Whang-Peng *et al.* 1982). A recessive locus on chr 13 may be involved in the genesis of ductal breast carcinomas and the loss of an allele on chr 5 may occur in colonic carcinomas (Lundberg *et al.* 1987; Solomon *et al.* 1987).

How can gene losses lead to tumour development? Comings (1973) has suggested that every cell contains structural 'transforming' genes, active during embryogenesis but suppressed during differentiation by dominant 'suppressor' or 'regulatory' genes. Loss of both copies of the latter may lift the suppression, with continuous expression of the transforming gene and tumour development as the result. Comings' theory is essentially consistent with the modern development, at least for retinoblastoma. Normally, the retinoblast differentiates into a retinocyte that has irreversibly lost the ability to divide. Children who inherit the deletion of one RB-1 allele from one of their parents run the risk of developing retinoblastoma only during their first years of life. If the second allele is not lost by a somatic change by the age of five, all retinocytes will have differentiated terminally. It is therefore likely that the wild-type RB-1 allele is essential for the terminal step, in a structural or a regulatory capacity.

Suppression by diffusible products of normal cells

Paul (1988) has recently summarized evidence indicating that small molecules, produced by normal cells, can diffuse in solid tissues through gap junctions and exert a damping effect on tumour cell precursors that contain activated oncogenes. If so, a second event may involve a reduction of the damping effect by modulating the gap junctions, or by creating a critical mass of transformed cells. Land *et al.* (1986) have shown that both *myc*- and *ras*-transformed rat fibroblasts can be suppressed by surrounding normal cells. Similar observations were made earlier by Stoker (1964) in relation to polyoma-transformed cells. Growth regulatory polypeptides that can inhibit the replication of certain cells, but may stimulate the growth of others have been demonstrated experimentally in several systems (Zarling *et al.* 1986; Newmark,

1987; Keski-Oja & Moses, 1987). They include an increasing number of known cellular products such as TGF-beta and members of the interferon family (Todaro, 1988).

Concluding remarks

It is widely accepted that tumour development and progression are due to sequential changes at the DNA level (for review see Klein & Klein, 1985). This is reflected by the 'reassortment of unit characteristics' at the phenotypic level (Foulds, 1954). Several of the currently known oncogenes can block specific steps in the maturation progress. Constitutively activated growth factors may inhibit maturation by urging their target cell to proliferate. Truncated growth factor receptors or faulty signal transducers may achieve a similar effect by emitting a continuous 'go' signal in the absence of external simulation. DNA-binding proteins like *myc* or *myb* may block maturation by interfering with the condensation of chromatin that is the hallmark of terminal differentiation in many cell lineages.

The category of genes described as anti-oncogenes, tumour suppressor genes, or emerogenes can antagonize tumorigenic behaviour at various levels. Somatic hybridization of normal cells with malignant ones has provided evidence that the normal genome may provide the tumour cell with the ability to respond to appropriate differentiation-inducing stimuli *in vivo*.

The differentiation block imposed by temperature sensitive v-*src*, a membrane associated tyrosine kinase, or v-*erb B*, a truncated growth factor receptor, may be permanently lifted by the temporary down regulation of the oncoprotein. Reexpression of the oncoprotein cannot halt or reverse the process; the potential 'go'-signal is apparently inactive when the cell has moved out from the sensitive 'maturation window'. Signals induced by physiological or chemical differentiation (or both) may down-regulate other highly expressed oncoproteins and thereby lift the maturation block. Such signals may even override the high expression of amplified *myc* genes.

Revertants fall into several categories. Deletion or mutational inactivation of viral oncogenes is relatively trivial. Cellular genes that can act beyond the level of oncoprotein expression are more interesting. They have been best documented in revertants isolated from *ras*-transformed fibroblasts. In the most extensively studied case (Bassin & Noda, 1987), the transformed and tumorigenic phenotype of v-Ki-*ras* infected fibroblasts was reverted by dominantly acting cellular gene or genes in spite of the continued presence of wild-type transforming virus and full expression of the p21 *ras* protein. The same gene(s) could also cancel the transforming action of *src*, *fes*, and all members of the *ras* family, but not *sis*, *fms*, *raf*, polyoma and SV40. This approach may provide new leads for a functional oncogene classification, based on suppressor sensitivity.

The oncogene terminology has evolved through a series of historical accidents and is actually a misnomer, but it is here to stay. It embraces a wide variety of genes that can influence the cell cycle at different levels. The consensus to collect a variety of normal genes under a common, cancer related name is based on the fact that they

may contribute to tumour development when they get out of hand. As long as this is so, we might just as well refer to the normal genes that can antagonize them in one system or another by the common name of 'emerogenes', as suggested by one of the originators of the oncogene terminology, George Todaro (1988) (*emero*: to tame, to domesticate). Some of them may control cell maturation. They, and/or other genes that act at different levels, may overrule the transforming action of highly expressed oncoproteins. They, or other genes that act at different levels, may overrule the transforming action of highly expressed oncoproteins. They or the normal alleles of the recessive cancer genes may also prevent tumours by obstructing the development of progression of preneoplastic cells. The study of the emerogenes is experimentally more difficult than the pursuit of the oncogenes, but it may turn out to be even more rewarding.

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