28

Chromosomes and Cancer: Inactivation of Tumor Suppressor Genes

Tumor/Nontumor Cell Hybrids: First Evidence for Tumor Suppressor Genes

Harris et al. (1969) showed that the fusion of normal and malignant cells produced hybrid cells that were generally nonmalignant. Rarely, however, a hybrid cell gave rise to malignant subclones. These always showed some loss of chromosomes derived from the normal parent, suggesting that one or more of these chromosomes carried a *tumor suppressor gene*. This has been confirmed by studies showing that the addition of a specific chromosome, chromosome segment, or single gene can suppress the tumorigenic capability of cancer cells. Thus, chromosome 11 suppresses the malignancy of Wilms renal tumor cells and of HeLa or other cervical cancer cells. The gene responsible has been mapped to 11q13, using deletions and translocations (Jesudasan et al., 1995). Another

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tumor suppressor locus has been mapped to 11p11.2–p12 using microcell hybridization and PCR analysis of a battery of genetic markers scattered along the chromosome (Fig. 28.1; Coleman et al., 1997). The normal function of a tumor suppressor gene is to prevent unregulated growth of cells. When both copies of such a gene are deleted or inactivated by mutation, the absence of the product allows uncontrolled growth to occur. An individual who has inherited a mutant tumor suppressor gene has a greatly increased risk of developing one or more types of cancer, because somatic mutation (deletion or inactivation) of only the single normal copy of the gene is necessary to abolish the tumor-suppressing function of the gene.

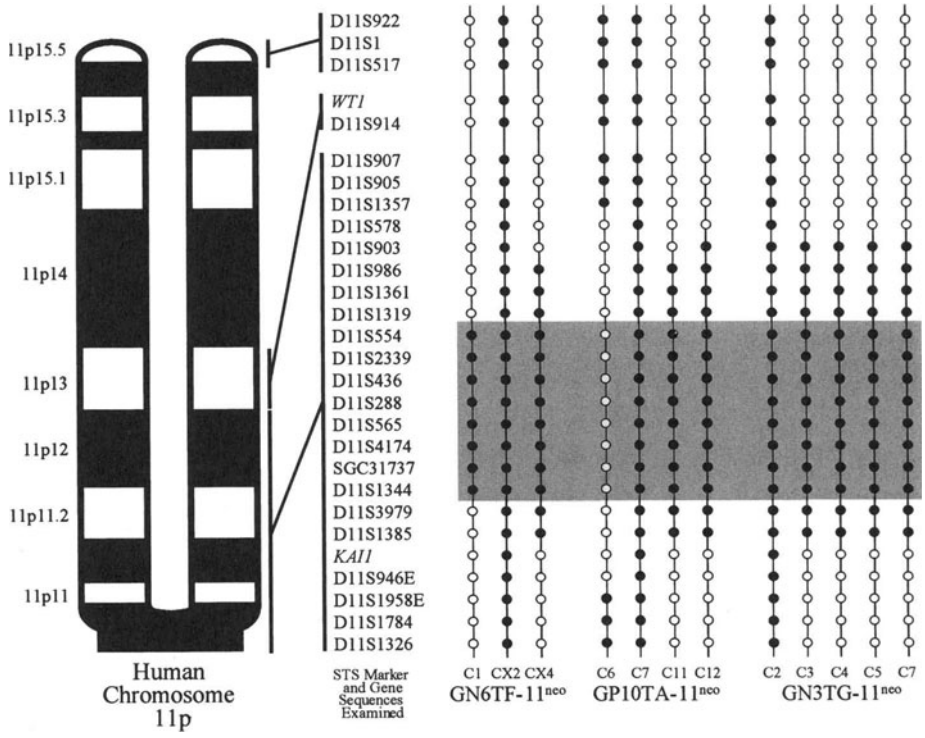

Figure 28.1. Summary of microsatellite PCR mapping in three microcell hybrid cell lines. Closed circles: retention of the marker; open circles: loss of the marker. The shaded area indicates the minimal liver tumor suppressor region (11p11.2–p14) identified using these three lines (courtesy of W. B. Coleman; based on data from three published studies and unpublished data).

Allele Loss and Loss of Heterozygosity

Loss of a chromosome segment carrying the wild-type allele from a cell heterozygous for a tumor suppressor gene leads to absence of its tumor-suppressing product. One mechanism for such loss of heterozygosity (LOH) is described in Chapter 24 and illustrated in Fig. 24.3. In normal cells, LOH occurs 10–100 times more frequently than point mutations (Tischfield, 1997). It is responsible for more than 90% of the renal cancers seen in von Hippel–Lindau syndrome (Gnarra et al., 1994) and more than 80% of familial breast and ovarian cancers (Neuhauser and Marshall, 1994). Studies have found that LOH of many specific chromosome regions is very common in many types of cancer. In addition, LOH analysis has shown consistent loss of the wild-type *BRCA1* allele in cancer cells from carriers of a *BRCA1* mutation, indicating that *BRCA1* is a tumor suppressor gene (Smith et al., 1992).

Retinoblastoma and the Two-Hit Model of Carcinogenesis

The most extensively studied of the cancers caused by inactivation of a tumor suppressor gene is the childhood tumor retinoblastoma, whose analysis led Knudsen to the two-hit model of carcinogenesis (Chapter 26). Retinoblastoma (RB) may occur as an isolated tumor, usually involving only one eye, or the tendency to it may be inherited, in which case there are multiple tumors, usually affecting both eyes. The genotype of a person with inherited retinoblastoma is either *RB/rb* or *RB/–*, where *rb* is a nonfunctional allele and—indicates a deletion of the locus. As Knudsen predicted, a somatic mutation or deletion of the remaining normal allele is required to produce malignant transformation of retinal cells. A small percentage of RBs arise in individuals with a constitutional heterozygous deletion of 13q14, historically the first evidence for the location of the gene (Chapter 1). Instances of LOH may be the result of chromosome loss (with or without duplication of the remaining chromosome), deletion, mitotic recombination, or gene conversion (Cavanee et al., 1983). Knowing its location made possible the positional cloning and sequencing of the gene, prediction of the amino acid sequence of the protein, isolation of the protein, and demonstration of the complete absence of RB mRNA or protein in RB cells.

In *RB/rb* or *RB/–* individuals, the risk of developing retinoblastoma is about 100,000 times higher than it is in the general population, and the risk for other types of cancer, especially osteosarcoma, is also increased. Many types of cancer are associated with inactivation of the gene, now called *RB1*, or its protein, pRB. Alternatively, many cancers instead show genetic alterations in proteins that regulate pRB, such as cyclin D, CDK4, or the p16 cell cycle inhibitor (Chapter 2). In over 90% of uterine cervical cancers, the RB protein is inactivated by binding to the E7 protein of the human papilloma virus (HPV), a major exogenous cause of cancer (Zur Hausen, 1996).

Mechanism of Tumor Suppression by a Functional *RB1* Gene

The RB protein acts as a negative regulator of growth and a tumor suppressor in two ways. It binds to the RNA polymerase I transcription factor upstream binding factor (UBF) and thus inhibits ribosomal RNA transcription (Cavanaugh et al., 1995). More important, pRB also arrests cell proliferation in the mid- to late G1 phase of the cell cycle by binding to E2F, a transcription factor that is essential for the G1/S transition and DNA replication (Chapter 2). The absence of a functional *RB1* gene thus leads to uncontrolled cell proliferation. Even when a normal *RB1* gene is present, pRB function may be impaired. For example, human papilloma virus E7 protein acts as an oncoprotein because it binds to pRB, causing it to release active E2F.

The two-hit model does not fully explain multistep carcinogenesis. Epstein-Barr virus infection early in life is a critical predisposing factor for both Burkitt lymphoma and nasopharyngeal carcinoma. Rearangements involving chromosomes 1, 3, 9, 11, 12, and 17 are common in these tumors, but none of the usual oncogenes or tumor suppressor genes have yet been implicated. Loss of heterozygosity for loci on chromosome 3 led Stanbridge and his associates to use microcell-mediated transfer of single chromosomes or chromosome fragments into a nasopharyngeal carcinoma cell line. The malignant phenotype was suppressed by the addition of any chromosome segment containing 3p21.3, suggesting the presence of an unknown tumor suppressor in that region (Cheng et al., 1998). The role of the virus may be to destabilize the genome, leading ultimately to inactivation of one or more specific tumor suppressor genes.

The p53 Tumor Suppressor Gene, *TP53*

Many tumor suppressor genes, like *RB1*, are involved in normal cell cycle control of proliferation (Table 28.1). Perhaps the most important of these for carcinogenesis is the p53 gene, *TP53*. Mutations in *TP53* are extremely common in cancers (Chapter 26). The p53 protein is essential for the G1 checkpoint that arrests cells with DNA damage in G1 until DNA repair can be carried out. The p53 protein is also required for apoptosis, by which cells with irreparable DNA damage are destroyed. Mutations in *TP53* foster carcinogenesis by both these mechanisms. If cells enter S with damaged DNA, they can produce daughter cells with mutations and chromosome breaks. Failure of apoptosis permits the generation of cells with progressively more mutations, and selection acting on these can lead to more malignant cells. Without apoptosis to destroy the damaged cells, a more malignant cancer can be generated by treatment with genotoxic drugs or radiation (Griffiths et al., 1997).

Most human tumors show abnormalities of both *RB1* and *TP53*, indicating that disruption of both pathways is usually necessary for tumorigenesis. Bates et al.

Table 28.1. Cell Cycle Regulators That Act as Tumor Suppressors

Gene	Product	Location
INK4C	p18	1p32
VHL	VHL	3p25
CIP1	p21	6p21
CDKN2A	p16, p19	9p21
INK4B	p15	9p21
*KIP2**	p57	11p15
ATM	ATM	11q22
KIP1	p27	12p13
RB1	pRB	13q14
TP53	p53	17p13
BRCA1	BRCA1	17q21
INK4D	p19	19p13

*An imprinted gene (maternal allele expressed)

(1998) showed why this is so. Loss of pRB frees E2F, which activates the *ARF* gene product, p14. This binds to p53-MDM2 complexes and prevents p53 degradation. This fail-safe mechanism of tumor suppression is disrupted if p53 is also mutated.

Other Genes That Affect the Cell Cycle

In addition to *RB1* and *TP53*, several other genes act as tumor suppressors through their inhibitory effects on the cell cycle (Table 28.1). A missing chromosome or band has often been the first clue to the localization of such a gene, but LOH analysis is also helpful. It can even detect submicroscopic deletions, which are very frequent in cancers.

Cyclin-dependent kinase (CDK) inhibitors arrest the cell cycle and thus control cell proliferation. The tumor-suppressing functions of pRB and p53 are also bypassed by mutations of the *ARF1/NK4a* (*CDKN2A*) gene locus at 9p21. These are almost as common as mutations in *TP53* itself (Kamb et al., 1994). This single locus produces two different gene products, p16/INK4A and p19/ARF (Fig. 28.2), by using separate promoters and two different reading frames, so that the resultant proteins have no amino acid sequence similarity. The p16 product normally inhibits CDK4 and CDK6 and thus blocks phosphorylation of the RB protein, maintaining pRB in its growth suppressor state (Monzon et al., 1998). The p19 protein is localized mainly to the nucleolus, but it leaves the nucleolus when pMDM2 binds to p53 and forms a larger complex with them. This blocks the nuclear export and degradation of p53 (Zhang and Xiong, 1999). Thus, mutations in *CDKN2A* can lead to a reduction in both pRB and p53 (Pomerantz et al., 1998). Many tumors show deletions that remove both copies of the *CDKN2A* gene. The deletions may be megabase pairs long. Many types of tumor show allele loss, as shown by LOH for marker loci in this region.

Mutations in the *BRCA1* gene are found in the majority of families with both breast and ovarian cancers (Easton et al., 1993). This gene appears to act as a tumor suppressor by activating the *WAF1/C1P1* gene product, p21, which blocks cell cycle progression into S (Somasundaram et al., 1997; see also Chapter 2). *BRCA1* is also required for one type of repair of oxidative DNA damage (Gowen et al., 1998).

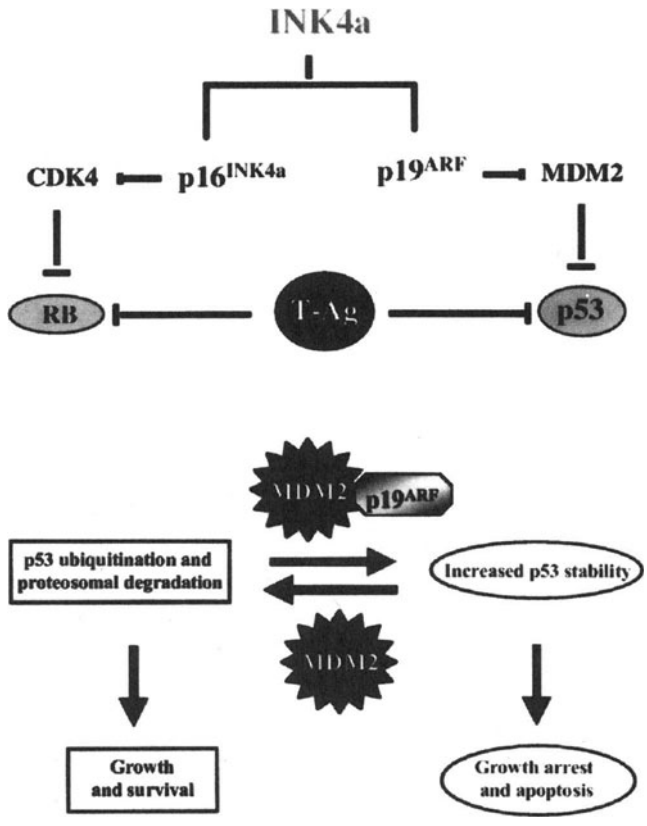

Figure 28.2. Two pathways affected by the two products of the *INK4A* gene, $p16^{INK4A}$ and $p19^{ARF}$. (A) Functional relationship of the two products with p53 and pRB. (B) Proposed mechanism for the enhancement of p53 functions by $p19^{ARF}$ (Pomerantz et al., copyright 1998, Cell Press).

Imprinted Tumor Suppressor Genes

Imprinted gene loci are those in which either the maternally or the paternally derived allele is inactivated, usually by methylation of the 5′ promoter or CpG island (Chapter 21). Cells therefore contain a single functional copy of each imprinted gene, and a *single* mutation is enough to abolish this function, in contrast to the *two independent* mutations necessary to abolish function of any non-imprinted autosomal gene. As a result, any imprinted gene with tumor suppressor activity may play a major role in some cancers. Breast tumors are one of the most

frequent types of cancer. One reason may be that the breast tumor suppressor gene *HIC-1* (hypermethylated in cancer-1) is imprinted in breast ductal epithelium (although not in other cell types), with one allele hypermethylated and the other allele unmethylated. *HIC-1* maps to 17p13.3, a region noted for LOH in breast cancer. Loss of heterozygosity of distal 17p, with loss of the unmethylated (functional) allele, was observed in 22 of 26 breast cancers with only a hypermethylated *HIC-1* gene or genes (Fujii et al., 1998).

A second imprinted tumor suppressor gene is *H19* (Chapter 21). *H19* RNA, which is not translated into a protein product, has tumor suppressor activity (Hao et al., 1993). It is interesting that several other tumor suppressor genes, including *RB1*, *VHL*, *p16/MTS*, *p15/INK4A*, *E-cadherin*, and *MLH1*, are hypermethylated in many types of cancer, although they are not known to be imprinted (Baylin et al., 1998). Hypermethylation inactivates these genes just as effectively as mutation or deletion, and this is found quite frequently in cancer cells. Loss of heterozygosity of 9p21, a region that contains the p16 tumor suppressor gene, is one of the most frequent changes in cancer. Cancer cell lines in which this has *not* occurred show methylation of the 5′ CpG island of the p16 gene, with complete transcriptional silencing that is reversible upon demethylation with 5-azacytidine. De novo methylation of this CpG island is seen in about 20% of primary neoplasms but not in normal tissues (Merlo et al., 1995).

Beckwith–Wiedemann syndrome (BWS) was described in Chapter 21. In BWS there is an increased risk of childhood tumors, including Wilms tumor, adrenal or liver carcinoma, and sarcoma. The few familial cases show autosomal dominant inheritance, with the disorder limited to those who inherited the mutation maternally. Linkage analysis in these families places the *BWS* gene at 11p15.5. Most cases are sporadic, with a normal karyotype, but a few involve a duplication, a translocation, or paternal uniparental disomy of chromosome 11, with the critical region 11p15.5. In normal kidney, only the maternal genes in this region are expressed, and the maternal 11p15 region tends to be lost from Wilms tumor cells. Microcell-mediated transfer of fragments containing 11p15 suppressed the tumorigenicity of Wilms tumor cells (Dowdy et al., 1991).

A candidate tumor suppressor gene in 11p15 is *KIP2*. Its protein product, p57, is an inhibitor of G1 cyclin-dependent kinases. Overexpression of p57 blocks the cell cycle in G1 (Chapter 2). In its absence, some cells will proliferate without control. *KIP2* is an imprinted gene: Only the maternal allele is expressed, the inactive paternal copy being fully methylated. There is reduced *KIP2* expression in Wilms tumor samples. The residual KIP2 protein presumably reflects the

presence of a few normal cells in the tumor samples analyzed. Confirmation that *KIP2* is indeed a tumor suppressor gene came from the demonstration that BWS can be caused not only by disruption or deletion but by an inactivating mutation of the maternally derived allele (Hatada et al., 1997).

Genes That Suppress Oncogenes or Influence Transcription

Colon carcinomas frequently overexpress *MYC* RNA and protein *without* the amplification or rearrangement of the gene discussed in Chapters 25 and 27. This appears to be the result of loss or inactivation of a tumor suppressor gene on chromosome 5, because introducing a normal chromosome 5 reduces the level of *MYC* expression and suppresses the malignancy of such colon carcinoma cells (Rodriguez-Alfageme et al., 1992). MYC functions only in the form of a heterodimer with a protein called MAX. Four proteins, the MAD/MXI1 family, compete with MYC for binding to MAX and thus block MYC. The genes for the four MAD/MXI1 proteins map to loci often disturbed in cancer cells. For example, *MXI1* maps to 10q24–q26 (Table 28.2), a region that often shows translocations or deletions in major kinds of cancer. In one study, half of prostate cancers showed a submicroscopic loss of one *MXI1* allele (LOH) and mutational inactivation of the retained allele (Prochownik et al., 1996).

SNF5 (Table 28.2) is a tumor suppressor gene with quite a different mechanism of action: It is involved in chromatin remodeling that fosters transcription (Chapter 5). Deletions of 22q11.2 are common in malignant rhabdoid childhood cancers of kidney, brain, and soft tissues. Homozygous deletions were found in 6 out of 13 of these tumor cell lines, and the shortest region of overlap contained the *SNF5* gene. In the other seven lines, there was a frameshift or nonsense (inactivating) mutation of one *SNF5* allele and loss of the other allele, leaving no functional copy (Versteege et al., 1998).

Chromosome band 10q23 is frequently deleted in endometrial, prostate, and breast carcinomas and in glioblastomas. Mapping homozygous 10q23 deletions led to the isolation from 10q23.3 of a tumor suppressor gene called *PTEN* (Table 28.2). The PTEN protein is a phosphatase whose target is phosphatidylinositol-3,4,5-triphosphate (PIP3), a key component of an important growth control pathway that stimulates proliferation and blocks apoptosis (Hopkin, 1998). The

Table 28.2. Normal Functions of Some Tumor Suppressor Genes

Gene	Location	Product*	Function regulated
KISS1	1q32–q41	KISS1	Metastasis suppressor
MSH2	2p22	MSH2	DNA mismatch repair
PMS1	2q31–q33	PMS1	DNA mismatch repair
VHL	3p25	VHL	Transcript elongation
MLH1	3p21–p23	MLH1	DNA mismatch repair
APC	5q21	APC	Cell adhesion
PMS2	7p22	PMS2	DNA mismatch repair
PTEN	10q23.3	PTEN	Signal transduction
MXI1	10q24–q26	MXI1	Inhibits MYC
H19	11p15.5	RNA only	Expression of nearby genes
MEN1	11q13	Menin	Transcription activation
BRCA2	13q12	BRCA2	Double-strand break repair
NF1	17q12	Neurofibromin	G-protein signal transduction
BRCA1	17q21	BRCA1	Double-strand break repair
DCC	18q21	DCC	Cell interactions
DPC4	18q21	DPC4	Signal transduction
SNF5	22q11.2	SNF5	Chromatin remodeling
NF2	22q12	Merlin	Signaling at membrane

*The letter symbols for the protein products are sometimes preceded by a "p," as in pVHL

PTEN protein has sequence similarity to the cytoskeletal protein tensin, which binds actin filaments. Overexpression of *PTEN* inhibits cell migration, spreading, and focal adhesion, suggesting that it may act as a tumor suppressor by negatively regulating cell interactions with the extracellular matrix (Tamura et al., 1998).

Mutations in the *MEN1* gene are responsible for familial multiple endocrine neoplasia type 1, an autosomal dominant disorder associated with tumors of the parathyroid, anterior pituitary, pancreas, and other neuroendocrine tissues. *MEN1* maps to 11q13 (Table 28.2), and LOH of 11q13 is seen in MEN1 tumors. As a result, the tumor tissue of affected individuals has lost the wild-type (normal) *MEN1* allele present in their normal tissues. Menin, the product of *MEN1*, interacts with the transcription factor JunD, blocking JunD–activated transcription (Agarwal et al., 1999).

Genes That Affect Cell Adhesion

Most normal cells are anchorage dependent, requiring attachment to an extracellular substrate for cell division. The reason for this is that anchorage is essential for the activation of cyclin E/CDK2 kinase in late G1 that initiates the G1/S transition (Chapter 2). In contrast, the kinase is always active in cancer cells, which are anchorage independent (Fang et al., 1996). This may indicate that normal cells produce a suppressor of cyclin E unless they are anchored to the extracellular matrix and that when inactivation or deletion silences the suppressor, cells can divide even if not anchored. Several of the cadherin genes have been mapped to regions that exhibit cancer-related LOH (Kremmidotis et al., 1998). E-cadherin is the main adhesion molecule in epithelia and is frequently absent in highly aggressive, invasive epithelial cancers. Restored expression of E-cadherin suppresses tumor invasiveness. *APC* (mutated in adenomatous polyps of the colon) may act as a tumor suppressor gene (Table 28.2) in two different ways. APC binds β-catenin, leading to its degradation. This blocks the activation of the Tcf4 transcription factor by β-catenin (Korinek et al., 1997). APC also interferes with spindle assembly by binding the EB1 protein that is normally found on microtubules (Su et al., 1995). Loss of APC function results in uncontrolled transcriptional activation of β-catenin/Tcf4 target genes.

Von Hippel–Lindau (VHL) syndrome is a rare autosomal dominant cancer predisposition disorder associated with both benign and malignant tumors, particularly of the kidney, pancreas, and brain. The *VHL* gene (Table 28.2) has been mapped to 3p25–p26 and identified (Latif et al., 1993). VHL patients are heterozygous for either a deletion or a point mutation of the gene, and renal carcinoma cells show allele loss on LOH analysis, indicating the absence of a functional copy (Crossey et al., 1994). The VHL protein (pVHL) acts in various ways. It is required for proper assembly of an extracellular fibronectin matrix. Since loss of this matrix is a feature of malignant cellular transformation, this may be the major tumor suppressor function of pVHL (Ohh et al., 1998). The region of pVHL that binds elongin B and elongin C is a hotspot for mutation in VHL kindreds, suggesting that the involvement of pVHL in the elongation of RNA transcripts may account for its role in tumor suppression, although the mechanism is unclear. It may be more important that the *CUL2* gene product, cullin, binds to the pVHL/elongin B/elongin C complex and is transported into the nucleus, where it plays a role in targeting cell cycle proteins for degrada-

tion. Failure to degrade these could lead to uncontrolled cell proliferation (Pause et al., 1997).

Metastasis Suppressor Genes

Microcell-mediated transfer of a normal chromosome 6 into malignant melanoma cells suppresses their ability to metastasize by at least 95% but does not affect their tumorigenicity, the ability to proliferate into a tumor at the site of injection of some of the cells into an immunosuppressed animal host. Lee et al. (1996) used subtractive DNA hybridization to detect any differences in the expression of mRNAs in the metastatic and nonmetastatic cells and isolated a novel gene, called *KISS1* (Table 28.2), from the nonmetastatic cells Transfection of a full length *KISS* cDNA into cancer cells could also suppress their metastasis. Surprisingly, FISH mapped *KISS1* to 1q32–q41, indicating that chromosome 6 carries a different metastasis suppressor gene, whose expression is required for *KISS1* expression.

DAP kinase mediates cell death (apoptosis). Some metastatic lung cancers have a reduced level of DAP kinase. The introduction of a functional *DAP* gene into cancer cells blocks their ability to metastasize and increases their sensitivity to apoptotic stimuli of the sort metastasizing cells are exposed to (Inbal et al., 1997).

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