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【Review】

Tumor Suppressor Genes, A New Era in Cancer Genetic Discoveries

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The search for cancer causing genes has taken several turns in the 20th century. Breakthrough during each turn has revealed major control mechanisms in multiple levels of cell growth regulation. First, following the discovery of Rous sarcoma virus (Rous, 1911) and src oncogene of the virus, eucaryotic counterparts of oncogenes, called proto-oncogenes, were identified (Stehelin *et al.*, 1976). These normal proto-oncogenes undergo mutation and gain additional activities which promote cell proliferation. Activated proto-oncogenes are frequently identified in human cancers, specifically at later stages of tumor progression. Secondly, mutational inactivation of tumor suppressor genes has been shown recently to be a major cause of human cancer. These discoveries mainly come from studies of rare familial cancer syndromes.

Retinoblastoma susceptibility (RB) gene was the first tumor suppressor gene cloned and its mutation has been shown in both hereditary and sporadic retinoblastoma, a rare malignant cancer of the retina in children. In the hereditary cases, the first mutation is inherited through germ-line, and the second mutation occurs somatically. In sporadic cases, both mutations occur in the retina cells. Work in our and many other laboratories in the past ten years has revealed the molecular mechanism of tumor suppression function of the RB gene. The retinoblastoma protein is a nuclear phosphoprotein that undergoes cyclic phosphorylation during the cell cycle (Lee *et al.*, 1987, Chen *et al.*, 1989). RB regulates cell cycle G1 to S progression (Goodrich *et al.* 1991). Its activity to block a cell at the G1 phase is at least partially due to its binding to E2Fs that results in repression of the E2F activities. E2F is a member of multiple gene family proteins whose transcriptional activation function is required for the expression of S-phase genes (Nevins, 1992; La Thangue, 1995). This regulatory mechanism is conserved as demonstrated by the discoveries of *Drosophila* E2F gene. In *Drosophila* embryos, the first G1 phase occurs after 16 cell divisions, and E2F-null *Drosophila* mutants fail to enter S phase in the 17th cell division (Duronio *et al.*, 1995). Unphosphorylated RB interacts and represses the activities of E2F (Shan *et al.*, 1992). Phosphorylation of RB by cyclin D-CDK4 or cyclin E-CDK4 kinase complexes leads to the dissociation of RB-E2F complexes and allows the progression from G1 to S phase (Riley *et al*, 1994). It is conceivable that inhibitors of cyclin-CDK kinase will also block the cell cycle progression. Indeed, recent identification of inhibitors of CDK (CKI, cyclin-dependent kinase inhibitors) has revealed the importance of CKI not only in cell cycle checkpoint controls but also in cancer predisposition (reviewed by Sherr and Roberts, 1995). One of the CKI, p16, has been found to be mutated in affected individuals of familial melanoma as well as many other tumor types (Table 1). Therefore, studies of retinoblastoma and melanoma clearly indicate that disturbance of cell cycle regulatory protein can lead to cancer predisposition. It is interesting that germinal mutation of RB or p16 results in different tumors although both are ubiquitously expressed in adult tissues.

Studies of the RB gene lead to further understanding of the cell cycle control. As more tumor suppressor genes are being identified, divergent functions emerge. A typical search for tumor susceptibility genes involves multiple laborious steps: initial linkage analysis to map the disease gene locus, then extensive physical mapping of the region, and finally identification of germ-line mutation in the affected individuals. Search of this kind has led to the cloning of many tumor suppressor genes that are affected in many common adult cancers (Table 1). All susceptibility genes identified so far are tumor suppressor genes with a single known exception, the multiple endocrine neoplasia gene which is linked to germ-line mutation of a proto-oncogene RET (Santoro *et al.*, 1995). Function of the known tumor suppressor genes includes cell cycle regulation, cell cycle checkpoint control, signal transduction, and DNA damage repair.

Table 1. Known tumor suppressor genes associated with hereditary malignancies.

Gene	Location	Tumor predisposed	Expression	Protein product & function
p53	17p13	rhabdomyosarcoma breast tumor osteosarcoma brain tumor	ubi	53 kD transcriptional factor mediate cell cycle arrest and apoptotic responses
BRCA1	17q21	breast cancer ovarian cancer	ubi	~220 kD ring finger protein
BRCA2	13q12-13	breast cancer	?	~370 kD protein
RB	13q14	retinoblastoma osteosarcoma pituitary tumor in mice	ubi	110 kD nuclear protein negative regulator of cell cycle
WT1	11p13	Wilm's tumor nephroblastoma	res	45 kD transcriptional repressor with zinc fingers
NF-1	17q11	neurofibrosarcoma schwanoma glioma pheochomocytoma	ubi	370 kD protein with anti-Ras activities
NF-2	22q12	vestibular schwanoma meningioma	ubi	66 kD cytoskeleton-binding protein
VHL	3p25	Von Hippel-Lindau: hemangioblastoma renal cell carcinoma	ubi	24 kD nuclear protein inhibitor of transcription elongation
TSC2	16p13.3	tuberous sclerosis renal carcinoma in rat	ubi	~198 kD protein with regional homology to GAP3 gene
$p16^{INK4A}$	9p21	melanoma	ubi	16 kD cdk4 inhibitor
$p15^{INK4B}$	9p21	melanoma?	ubi	15 kD cdk4 inhibitor
APC	5q21	adenomatous polyposis coli	ubi	310 kD cytoplasmic protein
MSH2	2p22	hereditary nonpolyposis colon cancer (HNPCC) sporadic colon tumors	ubi	~100 kD protein homologous to a yeast mismatch repair gene *MutS*
hMLH1	3p21-23	HNPCC	ubi	~85 kD protein homologous to a yeast mismatch repair gene *MLH1*
hPMS1	2q31-33	HNPCC	ubi	~110 kD protein homologous to a yeast mismatch repair gene *PMS1*
hPMS2	7p22	HNPCC	ubi	~95 kD protein homologous to a yeast mismatch repair gene *PMS1*
AT	11p22-23	lymphoma	ubi	homology to PI3 kinase
XP	-	skin cancer	ubi	nucleotide involved in excision repair

ubi, ubiquitous expression; res, restricted expression.

Mutation in mismatch repair gene is found to associate with colon cancer (Modrich, 1994). On the other hand, cells from patients with the skin cancer disease xenoderma pigmentosa are deficient in nucleotide excision repair (Sancar, 1994). These patients are extremely sensitive to sunlight exposure. The study underlines effects of environmental (i.e. UV) and metabolic (i.e. free radicals) mutagens as causative agents of cancer. Other agents, such as hepatitis B virus and papillomavirus, have long been known to associate with hepatoma and cervical carcinoma. Studies of the E6 and E7 oncoproteins of papillomavirus have revealed that part of the mechanism the viruses employed to transform cells is to disable of p53 and RB using their oncoproteins (Cheng *et al.*, 1995).

Recent studies, therefore, have linked cancer formation to aberrant expression of specific cellular genes. Mutation is a major cause leading to aberrant expression. Most interestingly, studies of sporadic breast cancer indicate that a novel mechanism may be used to disable the normal function of tumor suppressor genes. While breast cancer is

prevalent in developing countries, only 5-10% of breast cancer can be contributed to genetic predisposition. Recent studies of these predisposed families lead to discoveries of BRCA1 and BRCA2 genes (Miki *et al.*, 1994; Wooster *et al.*, 1995). Although the BRCA1 gene appears to be normal in sporadic breast cancer, mislocalization of the protein product has been shown in a high percentage of tumor samples (Chen *et al.*, 1995). Therefore, a novel mechanism other than mutation may be related to cancer formation.

One of the most studied tumor suppressor genes is p53. It is frequently mutated in multiple tumor types. In addition, germ-line mutation of the gene is found in patients with Li-Fraumeni syndrome that is associated with a high risk of breast cancer, sarcoma, etc. In response to DNA damage or stress condition, induction of p53 protein level is found, which in turn leads to the increase of p21, a universal CKI, resulting in cell cycle arrest. On the other hand, p53 can also induce the expression of the apoptotic gene bax (White, 1996). Therefore, mutational inactivation of p53 not only results in the loss of cell cycle checkpoint control but also a decrease in apoptosis, both contributing to deregulated cell growth.

Functions of known tumor suppressor genes provide a satisfying explanation of the well-known cancer characteristics, clonal origin and genetic instability. Mutations of cell cycle regulatory genes could directly lead to increased growth rate, facilitating clonal expansion, and could indirectly lead to genomic instability by allowing cell division to proceed in the presence of damaged DNA. On the other hand, mutations in DNA repair genes may lead to failure of repair of activational mutation of the proto-oncogenes or inactivational mutation of the tumor-suppressor genes, and subsequently result in uncontrolled cell growth.

Many animal models for studying tumor suppressor genes *in vivo* have been established. For example, p53-and p16-deficient mice develop normally but are highly susceptible to multiple tumor types (Downhower *et al.*, 1992; Serrano *et al.*, 1996). On the other hand, RB heterozygous mice predispose to pituitary tumor of the intermediate lobe at 100% penetrance (Lee *et al.*, 1992; Hu *et al.*, 1994). These model systems not only allow studies of the biological function of the genes, but also provide settings for novel approaches in cancer intervention. It is our hope that replacement of the tumor suppressor genes or molecules that mimic their function will eventually provide a better alternative than conventional therapies.

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抑癌基因研究的新發展【綜論】

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摘　要

經過最十年來的研究，生物學家總共發現了近二十種抑癌基因。主要的突破來自於一些家族性癌症：從罕見的兒童腫瘤到成人的結腸癌或乳癌。視網膜母細胞瘤抑癌基因的發現開闢了抑癌基因研究的新前景。目前我們對這基因的功能已有相當的了解，此基因的蛋白質產物可以抑制細胞週期由G1進入S期，從而抑制細胞的增殖。各個抑癌基因具有不同的功能，對這些基因作用的研究，使我們對腫瘤細胞的兩大特性－癌症起源于單一個癌變的細胞和癌細胞的遺傳物質不穩定性－有了探入的了解。近年來產生的各種基因突變的動物模型不但可以用以進一步探討這些抑癌基因的功能，而且是預防和新法治療癌症的最理想的實驗模型。

關鍵詞：抑癌細胞基因、細胞週期