PATHOLOGY ONCOLOGY RESEARCH
Vol 1, No1, 1995

Science Press Publishing Ltd. TUDOMÁNY KIADÓ

REVIEW

Recessive Oncogenes: Current Status

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Cell growth is under the control of a variety of positive and negative signals. An imbalance of such signals results in deregulation of cell behavior. Recessive oncogenes or tumor suppressor genes, opposite to dominant oncogenes, encode important cellular proteins which could function as negative regulators of the cell cycle, i.e., cell cycle brakes. Inactivation of recessive oncogenes, by allelic deletion, loss of expression, mutation, or functional inactivation by interacting with oncogene products of DNA tumor viruses or with amplified cellular binding proteins, will lead to uncontrolled cell growth or tumor formation. Besides the classic suppressor genes such as the p53 and RB, a growing number of novel tumor suppressor genes have been identified in recent years. While some tumor suppressor genes have been found to be important for the development of a large number of human malignancies (e.g., the p53 gene), others are more tumor type-specific (e.g., the NF-1 gene). Many human cancer types showed abnormalities of multiple tumor suppressor genes, offering strong support to the concept that tumorigenesis and progression result from an accumulation of multiple genetic alterations. In this review, we will begin with an overview (gene, transcript, protein and mechanisms of action) of the tumor suppressor genes (the RB, p53, DCC, APC, MCC, WT1, VHL, MST1, and BRCA1 genes) identified to date and then discuss the specific involvement of tumor suppressor genes in human malignancies including prostate cancer. Various chromosomal regions which potentially may contain tumor suppressor genes also will be reviewed. (Pathology Oncology Research Vol 1, No1, 7–22, 1995)

Key words: oncogenes, cell cycle, heterozygosity, mutation

Introduction

Multiple and sequential genetic damage to the cell are central to tumorigenesis. In 1982, the first molecular change in tumorigenesis, i.e., a mutation resulting amino acid subtitution (Gly to Val) in codon 12 of the ras oncogene in a human cancer cell line, was identified. Subsequently, there have been many further examples of genetic changes in tumors that have been documented at the molecular level.[1] These include point mutations, deletions, chromosomal translocations and gene amplification. Oncogenes are positive regulators of the cell cycle, as a result, activation of oncogenes by mutation or other mechanisms may lead to deregulated cell growth. To date, a large number of proto-oncogenes involved in various cellular signaling pathways implicated in human cancer have been identified.[1,2] The oncoproteins come from different classes of molecules and have been shown to function cooperatively in various pathways to regulate cell behavior.[1,2]

Historically, the existence of recessive oncogenes or tumor suppressor genes have been documented from several distinct lines of work, i.e., somatic cell hybridization experiments, microcell (containing single chromosome) transfer experiments and karyotypic analyses, as well as epidermological and statistical studies (see 3, 4 for reviews). Knudson postulated in 1971 that the rare childhood eye tumor, retinoblastoma, is triggered by two successive lesions in the genome.[5] He proposed that in sporadic retinoblastoma both lesions are sustained in the retinal cell as somatic mutations and in familial retino-

Received: March 20, 1995; accepted: May 13, 1995

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blastoma, one of the two mutations is acquired as a germline mutation, and the second mutation occurs as a somatic event.[5] Supporting this "two-hit" theory, studies in retinoblastoma led to prove that the two elusive genetic targets were the two copies of the 13q14-associated RB gene, and that the two mutational events involved the inactivation of both functional copies of this gene (see 3, 4 for reviews). Tumorigenesis occurs when both copies of tumor suppressor genes such as the RB genes have been inactivated. If one of a pair of critical homologous genes is inactivated by a mutation, then loss of the remaining normal gene via the development of homo- or hemizygosity for that gene, should result in malignancy. Loss of heterozygosity (LOH) studies have been instrumental in mapping commonly deleted chromosomal region(s) which may harbor potential tumor suppressor genes (see below for more details). To date, several other tumor suppressor loci have been identified through LOH, e.g., 1q for breast carcinoma, 11p for Wilms tumor, lung carcinoma, transitional cell bladder carcinoma, 11q for multiple endocrine neoplasia (MEN) type I, 17p for small cell lung carcinoma, colon carcinoma, osteosarcoma and astrocytoma, 8q for prostate cancer, 9p for melanomas, and 17q for NF type I and breast cancer[6-8] (and see below for details). The recently developed positional cloning techniques allow rapid identification of new recessive oncogenes, e.g., the DCC, MST1, APC, MCC, BRCA1 genes *(Table 1; see below for details)*.

Retinoblastoma gene (RB)

The RB gene encompasses 180 kb of DNA on chromosome 13q14, and encodes a 105 kD nuclear phosphoprotein (Rb). The unphosphorylated form of Rb is able to bind and inactivate the transcription factor E2F.[9] E2F can bind to a number of host cell promoters and appears to mediate mitogen-induced cell cycle progression from G1 to S phase *(Fig. 1)*. Therefore, the unphosphorylated Rb is a negative regulator for cell cycle. In tumor virus-transformed cells, human adenovirus E1A, SV40 large T antigen, and human papillomavirus (HPV) E7 can form complexes with the host cell unphosphorylated form of Rb.[10,11] By targeting Rb for complex formation, these viral proteins inactivate its function by releasing E2F from the Rb-E2F complex, and therefore promote cell growth. Two potential targets for control by E2F-Rb are the c-myc gene and the cdc2 gene. Rb can repress the transcription of the c-myc, N-myc, cdc2, thymidine kinase, DNA polymerase A, and dihydrofolate reductase (DHFR) genes, which are dependent on E2F sites in the responsive promoters.[12-14] Furthermore, Weintraub et al.[15] provided evidence that Rb protein switches the E2F site from a positive to a negative element. They showed that E2F sites act as negative elements which inhibit the activity of other promoter elements in the presence of Rb, but are positive elements in the absence of an active form of Rb. E2F-site activity oscillates between a positive and a negative

Table 1. **Tumor Suppressor Genes**

Gene	Chromosomal localization	Cellular localization	Action	Organ or tissue origins or tumor
RB	13q14	nucleus	regulates gene activity and cell cycle	eye, bone, breast, lung esophagus, prostate, bladder, kidney, cervix
p53	17p13.1	nucleus	regulates gene activity and cell cycle	breast, colon, bladder, ovary, brain, testis, soft tissues, kidney, skin, blood, lung, pancreas, stomach, esophagus, liver, prostate
DCC	18q21.3	cell membrane	cell adhesion and signal transduction	colon, stomach, mouth, esophagus, uterine, blood, testis, prostate pancreas
APC	5q21	cytosol	promotes microtubule assembly	colon, lung, stomach, esophagus, mouth, breast, ovary, prostate
MCC	5q21	cytosol	unknown	colon, lung, stomach, breast, esophagus, ovary, prostate
WT-1	11p13	nucleus	regulates gene activity	kidney, bladder, eye, soft tissue, lung, ovary
NF-1	17q11	CM-associated	regulates p21 (ras) signaling pathway	nerve, bone, skin
NF-2	22q12	CM-associated	unknown	nerve, colon, breast, skin
VHL	3p25	CM-associated	unknown	CNS, eye, kidney, lung, breast, ovary, skin, soft tissue
MST1	9p21–22	nucleus	inhibits CDK and regulates cell cycle	skin, esophagus, pancreas, nose, lung, bladder, kidney, brain, blood, soft tissue
BRCA1	17q21	nucleus	transcription factor?	breast, ovary, prostate

CM = cell membrane; CNS = central nervous system

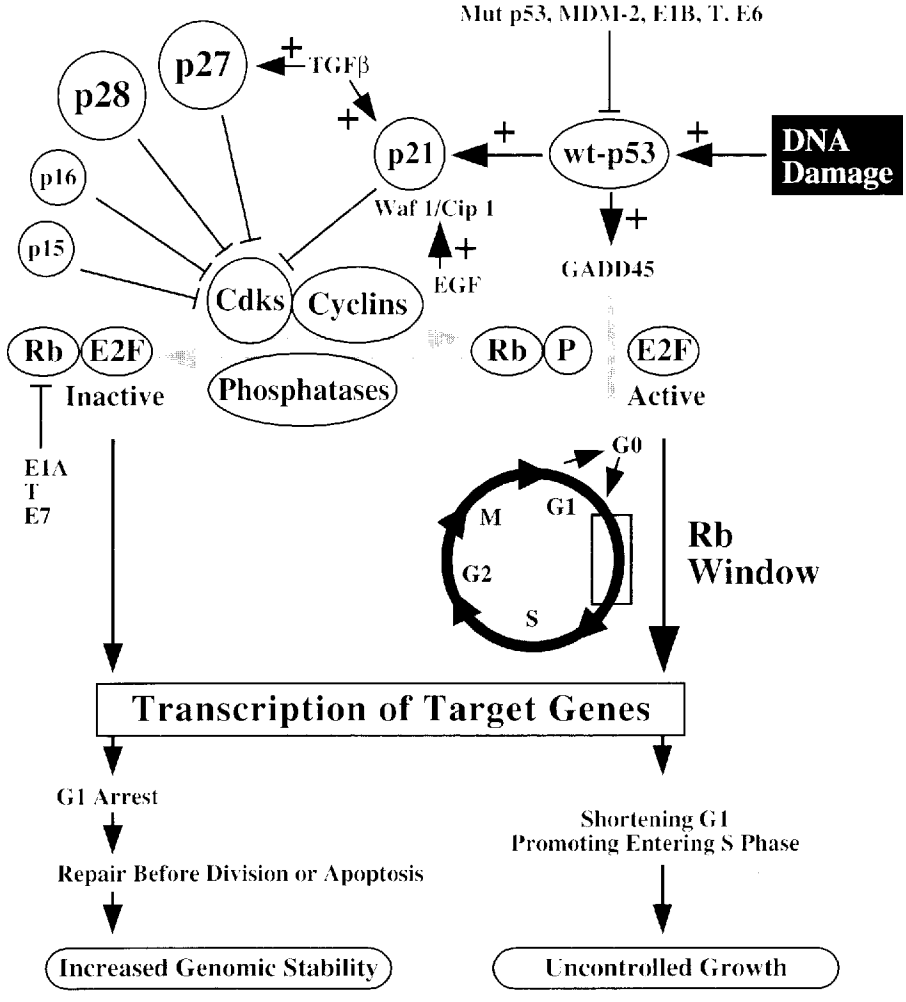

Figure 1. Participation of tumor suppressor genes in cell cycle regulation: The p53-RB signaling pathway. Upon DNA damage, cells rapidly increase wild-type (wt) p53 protein at posttranscriptional level. Induction of wt p53 leads to transcriptional activation of p53-dependent genes such as WAF1 (p21), MDM-2, and GADD45. The role of GADD45 in mediating p53-dependent responses is still elusive. MDM-2 is involved in the autoregulatory loop of p53-MDM-2. p21 (Waf1/Cip1), as well as p16, p27 and other CDK inhibitors, will inhibit the activation of cyclin-CDK complexes. p53-independent induction of p21 by EGF and TGFb1 have been reported. p27 is induced by TGFβ and cell-cell contact. Activation of the cyclin-CDK complexes is required to phosphorylate Rb protein and the phosphorylated Rb protein will in turn release E2F, a transcriptional factor originally complexed and inactivated by unphosphorylated Rb protein in G1 phase of the cell cycle. Released E2F activates the transcription of genes required for transition from G1 to S phase. Therefore, p53 and Rb proteins in normal cell function at different levels of the same signaling pathway to mediate DNA-damage-induced G1 arrest. Loss of function of p53 and/or Rb proteins by mutation, loss of expression, interaction with viral oncoproteins and cellular-inactivating protein(s) will result in genomic instability and uncontrolled cell growth. In some cells, p53 induction by DNA-damage seems to induce apoptosis rather than G1 arrest. In these cases, loss of apoptotic signals also may result in genomic instability. Red lines: stimulation; Blue lines: inhibition; Rb: retinoblastoma protein; GADD45: growth arrest and DNA damage; TGFβ: Transforming growth factor β; EGF: Epidermal growth factor.

element according to the phosphorylation state of Rb during the cell cycle.

The state of phosphorylation of Rb is controlled by a group of cyclin-dependent kinases (CDKs) and a group of phosphatases, and the CDK activity is in turn governed by the balance of cyclins and CDK inhibitors (e.g., p15, p16, p18, p21, p27, and p28) *(Fig. 1 and 2;*[17] see 16 for review). Therefore, the Rb protein is an inhibitor of cell cycle progression at G1/S by its inhibition of the E2F transcriptional factor family. In fact, E2F overexpression promotes DNA synthesis in growth-arrested cells.[18]

The importance of the RB gene in the development of human malignancies has been documented in retinoblastoma, breast cancer, esophageal cancer, osteosarcoma, lung, bladder, renal cell, cervical carcinomas[19] (see 20 for reviews), and prostate cancer[21,22] (and see detailed discussion below).

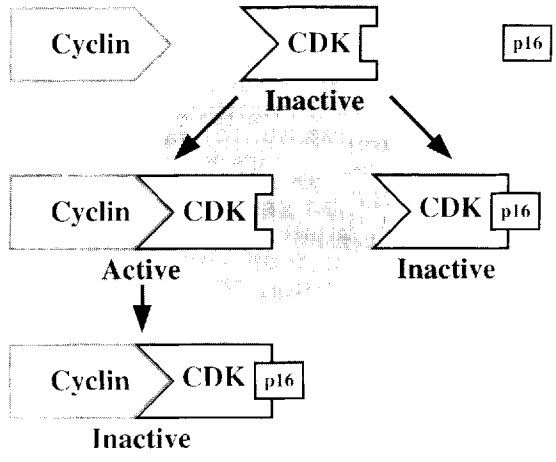

Figure 2. Inhibition of cyclin-depedent kinase (CDK) activity by p16. Free and inactive cyclin-dependent kinase (CDK) is activated by complexing with cyclin. Binding of p16 to the activated cyclin-CDK complex leads to functional inactivation. Since CDK activity is required for the G1 to S phase transition of the cell cycle (see text for details), p16, as an inhibitor of CDK, is a signal for cell cycle G1 arrest.

Transfection of the RB gene has been shown to suppress the tumorigenicity of human prostate cancer cell lines.[21] Abnormalities of the RB gene have been identified in a human prostate cancer cell line as well as prostate cancer tissues.[21,22] A deletion of 105 bp in exon 21 of the RB gene has been demonstrated in DU 145 cells. Such a deletion cause a shortened mRNA and a dysfunction of RB gene product.[21] A 103 bp deletion of RB promoter sequences was identified in a small cell carcinoma of the prostate. Loss of the regulatory sequences in the RB promoter has been demonstrated to alter RB gene expression and function.[22] However, in another study such deletions have not been found in 23 cases of prostatic adenocarcinoma and one case of small cell carcinoma of the prostate.[23] Phillips et al. studied LOH of the RB gene using two intragenic probes, i.e., p68RS2.0 (Rsa I) and p123m1.8 (Bam H 1), found that six (67%) of nine informative cases had allelic deletion at the RB locus.[24] This result is not consistent with the previous observations from Carter et al. where the same polymorphic site(s) was studied.[25] Probing at the Rsa I RFLP site, Carter et al.[25] detected a LOH rate of 23% (3/13) in prostate cancer. We screened a number of prostate cancer cases for LOH of the RB gene using polymerase chain reaction (PCR) with primers flanking two (Rsa I and Bam H 1) RFLP sites and one VNTR site. Our results are similar to that obtained by Carter et al.[25] (X Gao and KV Honn, unpublished observations).

p53 Gene

The p53 gene was discovered through the ability of its encoded protein to complex with the SV40 large T antigen in virus-transformed cells. The p53 protein of the normal cell is metabolically unstable and only accumulates to very low steady-state levels. Upon complexing with SV40 large T antigen, turnover of p53 is virtually eliminated, and it accumulates to levels often 100-fold higher than normal. This was thought to be at least part of the the cause of transforming effects of SV40 large T antigen. When wild-type p53 cDNA was used in such transfection experiments, it was found to be strongly growth-inhibitory. Wild-type p53 can suppress or inhibit the transformation of cells in culture by either viral or cellular oncogenes. Additionally, while the wild-type p53 possesses tumor-suppressing activities, the mutated p53 is oncogenic (see 26 for a review). Furthermore, mutated p53 has been shown to block wild-type p53-mediated signal transduction (to be discussed in more detail below).

The p53 gene, located in a 20 kb fragment of chromosome 17p13.1, and contains 11 exons. The fully processed and spliced mRNA is 2.2-2.5 kb in size and is ubiquitously expressed. Wild-type p53 has been shown to be a suppressor of cell growth and transformation,[27] causing a G1 block in cell cycle progression[10,28] and in certain cell types inducing apoptosis.[29,30] Mutations in the p53 gene have been demonstrated to be the most common genetic alterations in human cancers.[26] Functional inactivation may result from genetic aberrations within the p53 gene, most frequently missense mutations, or inactivation by interacting with viral and cellular oncoproteins (please see 26, 28, 31 for reviews. Loss of wild-type p53 function leads to deregulation of the cell cycle checkpoint and DNA replication, defective or inefficient DNA repair, selective growth advantage and, as a result, tumor formation and progression.[26,32]

At least one of the mechanisms which have been suggested to be the cause of the p53-mediated growth suppression is the ability of p53 to function as a transcriptional factor. p53 has been found to suppress a number of genes with TATA-element-containing promoters[33,34] and this suppression seems to be sequence-independent and involve p53 binding to the basal transcription components (e.g., TATA-binding protein or other transcriptional factors).[35,36] Recently, wt-p53 also has been demonstrated to suppress the promoter activity of the human immunodeficiency virus type 1 (HIV-1) long terminal repeat in both in vitro and in vivo transcription assays.[34] The presence of the HIV-1 TAR sequence and oncogenic transactivator, Tat protein, inhibits wt-p53-mediated suppression of HIV-1 LTR activity. It is not known whether TAR or Tat binds to wt-p53.[34] On the other hand, sequence-specific DNA-p53 binding has been observed with a 20 bp consensus site, 5'-PuPuPuC(A/T)(T/A)GPyPyPy-3'.[30] It has been

demonstrated both in vivo and in vitro that p53 activates transcriptions from promoters bearing such sites.[40] Several genes contain p53-binding sites, including muscle creatine kinase, GADD45, MDM2, a GLN retroviral element, WAF1/CIP1 (p21), and bax genes.[41-45]

Three different DNA tumor viruses, (i.e., SV40, adenovirus, and human papillomavirus type 16 or 18), have evolved a mechanism to deal with the negative regulation by p53. The oncoproteins (SV40 large T antigen, adenovirus E1B, and HPV E6 protein) can bind and form complexes with p53 (see 10, 11, 28 for review). More importantly, viral oncoproteins, such as large T antigen and E1B, have been shown to inhibit or block the p53-mediated signal transduction, e.g., induction of p21 (WAF1/CIP1) expression and cell cycle G1 arrest.[10,28,40] The MDM2 gene encodes a protein of approximately 490 amino acids and was found to be amplified in sarcomas. Overexpression of the MDM2 can increase the tumorigenicity of NIH 3T3 cells and abolish the wild-type p53 restraint on cell growth. The cellular protein MDM2 can inactivate the p53 by binding to its ACT domain.[46]

Interestingly, all of the point-mutated p53 proteins analyzed demonstrate loss of this sequence-specific DNA-binding function and many of the mutant proteins can act dominant negative in a fashion to inhibit this activity of the wild-type p53 proteins.[39] Classically, this dominant negative function is achieved by the formation of inactive hetero-oligomers between the mutant and wild-type p53 protein, i.e., p53 probably binds to DNA as a tetramer. The oligomerization (function resides in the C-terminus) of p53 occurs outside the area where most mutations occur. Consistent with this model is the inhibition of DNA-binding activity of p53 by both SV40 large T antigen and by MDM2. Since T antigen and probably MDM2 are DNA binding proteins and transcription factors in their own right, the hetero-complexes may have changed their DNA-binding specificity rather than loss of function.

It also has been shown that exposure of cells to non-lethal dose of DNA damaging agents, such as ultraviolet light, γ-irradiation and cancer chemotherapeutic agents, causes a transient accumulation of normal p53 proteins via posttranslational mechanisms, mediating arrest of the cell cycle at the G1 phase and leading to the inhibition of replicative DNA synthesis.[28,47-49] p53 protein has been proposed to be a molecular guardian to monitor the integrity of the genome in normal cells.[50] If DNA is damaged, p53 accumulate and switches off replication to allow time for repair. If repair fails, p53 may trigger apoptosis.[50] Tumor cells in which p53 is inactivated by mutation, or by binding to host (MDM2) or viral proteins, cannot carry out this function. These cells are genetically unstable and will accumulate mutations and chromosomal rearrangements at an increased rate, leading to rapid selection of malignant clones. This model provides an explanation why DNA viruses replicate their DNA within the cell nucleus, but don't trigger the damage response since they need the cell to be in S phase for their own replication to occur. It is also consistent with the observation that p53 null mice develop normally but have a very high tumor incidence.[51] It is now clear that both Rb and p53 interact functionally at the cell cycle *(Fig.1)*. Control of E2F by Rb can regulate the transition of G1 to S phase. Transcriptional activation of $p21^{WAF1/CIP1}$ and a cyclin-dependent kinase inhibitor (CDKI) by p53 can inhibit CDKs and cell cycle progression.[43,44,52] When Rb function ($Rb^{+/+}p53^{+/+}$ or $Rb^{+/-}p53^{-/-}$) is intact, cell proliferation can be arrested in differentiating cells by functional inactivation of E2F by Rb. Since p53 can promote growth arrest as well and works upstream of the Rb protein in the signaling pathway *(Fig.1)*, the presence of p53 may be redundant and therefore not apparent. In the absence of functional Rb, the presence of wt p53 ($Rb^{-/-}p53^{+/+}$) induces apoptosis, whereas the absence of wt p53 ($Rb^{-/-}p53^{-/-}$) leads to uncontrolled cell growth and tumor formation.[50]

Mutations of p53 are the most common genetic alterations in human malignancies. It has been estimated that more than half of human cancers harbor p53 mutations[7,26] (and 53 for reviews). To date, p53 mutations have been found in cancers of the lung (both SCLC and non-SCLC), breast, colon, esophagus, liver, bladder, ovary, brain and sarcomas, lymphomas and leukemias[7,54] (see 55-59 for reviews:) as well as squamous cell carcinoma of the head and neck,[60] pancreatic carcinoma,[61] gastric cancer,[62] testicular seminoma,[63] transitional cell carcinoma and renal cell carcinoma,[64] and prostate cancer (see below for detailed discussion).

Since Issacs et al. demonstrated that transfection of the wt-p53 cDNA could suppress the tumorigenicity of human prostate cancer cell lines,[65] research on the role of the p53 in human prostate cancer development has been intensified.[51] However, the role of p53 in human prostate cancer is still unclear and remains a point of controversy. While a number of groups demonstrated high p53 mutation rate in prostate cancer, others reported rare mutations (see 66 for review). Such frequency differences of the p53 mutation in prostate cancer among various groups could partially due to the geographic or demographic factors.

The correlation between p53 abnormalities and prostate cancer progression have been reported in a number of studies including data from our group (see 66, 67 refs. therein for reviews). However, two studies suggested that p53 abnormalities may be an early event in prostate cancer progression.[68,69] Such controversy could only be resolved by investigation of larger number of patients. We have determined LOH of the p53 gene, levels of p53 and MDM2 expression, and mutations of the the p53 in 28 prostate cancer samples.[46] We have observed a high frequency of LOH and decrease in p53 expression in prostate cancer samples. In 18 cases which expressed similar amounts of p53 mRNA compared to their matched normal

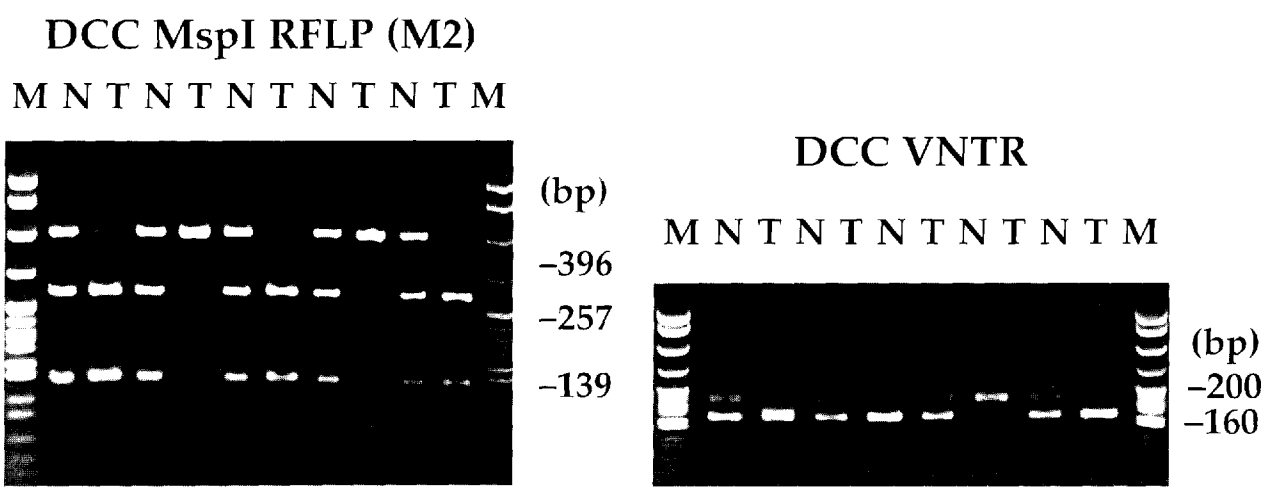

Figure 3. Detection of loss of heterozygosity (LOH) at the DCC locus by polymerase chain reaction (PCR) in human prostate cancer. Genomic DNA was isolated from matching prostatic carcinoma (T) and normal prostate tissues (N). PCR amplification was performed using primers framing a MspI RFLP (M2) site and a VNTR polymorphic site at the DCC locus. PCR products were digested (for Msp I RFLP) or not digested (for VNTR polymorphism) with the appropriate restriction enzyme and electrophoresed on 2.0-3.5% MetaPhor agarose (FMC BioProducts) or 6% polyacrylamide gels, which were then visualized and photographed. Representative samples of DCC-LOH at the MspI (A) and VNTR (B) sites are shown. Normal DNA from heterozygous patients showed three bands at the DCC locus after MspI digestion of the PCR products, i.e., 396, 257, and 139 bp bands. DCC-LOH was demonstrated when the tumor DNA retained either a 396 bp band or 257 and 139 bp bands only (A). At the VNTR site, a spectrum of alleles ranging from 150 to 210 base pairs in size was generated from PCR. Strong allelic imbalance was seen in carcinoma (T) compared to normal tissues (N), LOH at this site was judged when tumor tissues (T) loss one of the alleles detected in matching normal tissues (N) (B). The primers used were: 5'-GATGA-CATTT-TCCCT-CTAG-3' (VNTR sense), 5'-GTGGT-TATTG-CCTTG-AAAAG-3' (VNTR antisense); 5'-TGCAC-CATGC-TGAAG-ATTGT-3' (M2 sense), and 5'-AGTAC-AACAC-AAGGT-ATGTG-3' (M2 antisense). Negative controls without genomic DNA were performed for each set of PCR reaction. RFLP: restriction fragment length polymorphism; VNTR: variable number of tandem repeats; M: pBR322 MspI digest.

prostate tissues, 7 (39%) had MDM2 overexpression. p53 mutation at exons 5-8 was rare in patient samples but frequent in cell lines. Our results indicate that p53 abnormalities (allelic deletion, low expression, MDM2 overexpression and mutation) occur at a high rate during prostate cancer development and that the frequency of p53 alterations appears to correlate with tumor grade/stage.[46]

Deleted in Colon Carcinoma (DCC) gene

The DCC gene, which has 29 exons and spans approximately 1.4 Mb on chromosome 18q21.3, has been identified by positional cloning.[70,71] The predicted transmembrane DCC protein product has 1447 amino acids. The extracellular domain of approximately 1000 amino acids has four immunoglobulin domains and six fibronectin type III-like domains. The 325-amino acid cytoplasmic domain is unique, sharing no similarity to previously characterized proteins.[72,73] The DCC protein was found to be expressed in axons of the central and peripheral nervous system and in differentiated cell types of the intestine.[72] It has been reported that NIH3T3 cells expressing the DCC protein stimulate neurite outgrowth in rat PC12 pheochromocytoma cells.[74] Down regulation of DCC expression by antisense RNA transforms rat-1 fibroblasts.[75] Transfection of DCC cDNA into a tumor cell line lacking DCC expression can suppress tumorigenesis of the transfectants.[71]

The DCC gene has been reported to show allelic deletion in human colorectal, gastric, esophageal cancers, male germ-cell tumors, and endometrial carcinomas as well as primary leukemia.[70,76-79] and decrease or loss of expression in human oral, colorectal, pancreatic carcinomas and male germ-cell tumors.[70,79,80] However, mutations of the DCC coding sequence have only been observed in colorectal and esophageal carcinomas.[71,77]

Data obtained by our group strongly implicated the DCC gene in prostate cancer.[81] Human prostate cancer showed a high frequency of LOH (45%) and loss of expression (86%) of the DCC gene, suggesting a role for the DCC gene in the pathogenesis of this cancer.[81] The polymerase chain reaction-loss of heterozygosity (PCR-LOH) assay was used to detect allelic deletion of the DCC locus using two Msp I (i.e., M2 and M3) RFLP sites and one VNTR (or microsatellite) site and the reverse transcription-polymerase chain reaction (RT-PCR) utilized to measure DCC mRNA expression level.[81] Multiple polymorphic sites used for PCR-LOH increased assay sensitivity. The examples of DCC LOH detected by PCR are shown in *Fig.3*. The involvement of the DCC gene in

prostate cancer has been supported by a subsequent study where Brewster et al. demonstrated a DCC LOH rate of 26% in this cancer type.[82]

Adenomatous Polyposis Coli (APC) and Mutated in Colorectal Cancer (MCC) genes

The APC and MCC genes were isolated by positional cloning from human chromosome 5q21.[83,84,85] It has been shown that most mutant APC proteins are capable of binding to wild type APC protein and may inactivate it in a dominant negative manner.[86,87] The homodimer (APC/APC)[88] and heterodimer (APC/Catenin)[89] formation are mediated by the first 55 amino acids and a 27-amino acid residue fragment (residue number 1013-1039 or 1129-1174) containing a 15-amino acid repeat of APC protein, respectively. Recently, wt-APC but not mutant APC protein has been demonstrated to be able to associate with microtubules in vivo and to promote their assembly in vitro.[90,91] Transfection of a full length APC cDNA into a human colon cancer cell line has been reported to result in suppression of tumorigenicity.[92] However, the exact function of MCC protein has not yet been elucidated.

The known mechanisms of APC and MCC inactivation in human malignancies are loss of heterozygosity, mutations, and loss of mRNA expression (see 93 for detailed discussion). The APC gene has been implicated in familial adenomatous polyposis, sporadic colorectal cancer, and possibly in lung, gastric, pancreatic, esophageal, oral, ovarian, and breast cancers.[83,85,93-95] The MCC gene also has been suggested to play an important role in the pathogenesis of gastric, sporadic colorectal, esophageal, ovarian, breast and lung cancers.[83,84,93,94]

Recently, we demonstrated that 65% and 57% of prostate cancer tissues showed LOH and loss of mRNA expression, respectively, of the APC/MCC genes.[93] It has also been reported that three of the seven informative prostate cancer cases showed LOH at chromosome 5q21 when a probe (EF5.44) from a region within 1 centimorgan from the APC gene was used.[24] Although LOH and loss of mRNA expression data supported a positive role of the APC gene in prostate cancer, polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) failed to detect any functional mutations in 18 prostate cancer patients.[96] However, only the 5'-half of the exon 15 of the APC gene was targeted.[96]

Wilm's Tumor (WT-1) gene

Wilm's tumor is a childhood nephroblastoma with a genetic predisposition. The tumor may be associated with congenital malformations of the urogenital tract, aniridia, and mental retardation. The WT-1 gene, spanning about 50 kb at chromosome 11p13, has 10 exons.[97] The mRNA is about 3 kb in size, and several spliced forms and an antisense message have been identified.[98] The WT-1 proteins are 46-49 kD in size, contain four zinc finger motifs and are able to bind to specific DNA sequences,[99] suggesting that they could be transcriptional factors.[97] Indeed, the WT 1 protein binds to the early growth response-1 (EGR-1) site and represses transcription in transient transfection assays.[100] Four additional genes, i.e., the fetal mitogen insulin-like growth factor II (IGF-II) gene, the platelet-derived growth factor (PDGF) A chain gene, transforming growth factor (TGF)-β 1 and insulin-like growth factor (IGF) 1R gene, have been shown to be targets of transcriptional repression by WT 1 protein.[101-103] Alternative splicing within the DNA binding domain of the WT-1 gene generates proteins with distinct DNA binding specificities and probably different physiological targets for regulation.[104] Mapping studies demonstrate that amino acid residues 84-179 of WT 1 protein are required for transcriptional suppression, whereas amino acid residues 180-294 contain a domain mediating transcriptional activation.[101] The WT-1 gene demonstrates a very restricted pattern of expression, being found in mesenchymal buds, renal vesicle, and glomerular epithelium of the embryonic kidney, fetal testis and ovary, and some hemopoietic cells. Therefore the WT-1 protein has the characteristics of a tissue-specific transcription factor that is expressed only at certain developmental stages in contrast to the ubiquitously expressed RB gene. Thus, disruption of a gene that is active during a critical period in the development of a specific organ can lead to neoplastic growth in that organ.[105] LOH of 11p13 has been observed in Wilm's tumor,[106] bladder carcinoma,[107] and non-small cell lung cancer.[108] Naturally occurring mutations in the zinc finger region as well as other coding regions of the WT 1 gene have been found in both inherited and sporadic Wilm's tumors, Denys-Drash syndrome[109,110] (see 111 for review), ovarian cancer,[112] and mesothelioma.[113]

Neurofibromatosis Type 1 and 2 (NF-1 and NF-2) genes

NF-1 involves cells originating in the embryonic neural crest and creates a variety of benign growths and malignant tumors which include peripheral neuroblastomas, cafe au lait macules, optic nerve gliomas, and abnormalities of the bone. The NF-1 tumor suppressor gene spanning 350 kb on chromosome 17q11 was isolated and shown to encode a protein (termed neurofibromin) sharing structural similarity with three proteins that interact with the products of the ras proto-oncogenes.[114,115] The structural similarities suggest that NF-1 participates in a much-studied signaling pathway triggered by the p21 ras proteins. NF-1 may act as a pure down-regulator of p21 ras and block ras-mediated mitogenic signaling.[4,116] Large chromosomal rearrangement and mutations in both translated and untranslated regions of the NF-1 gene have been identified in tumors in NF-1 patients.[117,118] However, to

date, there is no evidence in the literature to suggest that alterations of the NF-1 gene exist in tumors other than those of NF-1 patients.[117-119] NF-2 is associated with the development of schwannomas, meningiomas, and ependymomas. The NF-2 gene, flanking a region of 35-45 kb on chromosome 22q12, has been identified and the cDNA cloned.[120,121] The open reading frame (ORF) of the full-length NF-2 cDNA is 1.8 kb in size and the deduced amino acid sequence of the protein product, termed schwannomin, has homology with proteins at the plasma membrane and cytoskeleton interface. The exact function of schwannomin is still unknown at the present time. Both germline and somatic mutations resulting in amino acid substitution, frame shift and splicing error, have been found in NF-2 patients.[120] LOH of chromosome 22 and mutations of the NF-2 gene have been reported in a group of tumors not seen frequently in NF-2 patients, e.g., gliomas, pheochromocytomas, melanomas, and colon and breast cancer.[120,122]

von Hippel-Lindau Disease (VHL) Gene

von Hippel-Lindau (VHL) disease is a dominantly inherited cancer syndrome predisposing affected individuals to a number of tumors, which include hemangioblastomas of the central nervous system and retina, renal cell carcinoma, and pheochromocytoma. The VHL gene, spanning nearly 80 kb at chromosome 3p25, has been identified by positional cloning.[123] The transcripts of two distinct sizes were found to be expressed in a tissue-specific manner and may represent alternatively spliced forms of VHL mRNA in human as well as other species.[123,124] The partial deduced cDNA sequence demonstrates a degree of homology with an acidic repeat domain of a procyclic surface membrane glycoprotein in Trypanosoma brucei.[123] The function of the VHL protein has not yet been elucidated. Chromosomal rearrangement, deletion, and somatic and germline mutations have been found not only in cancers of the VHL disease families[123,125-127] but also in sporadic renal cell carcinoma,[128] cancers of the lung,[129] breast and ovary,[130] mesothelioma,[131] head and neck cancer,[132] and CNS hemangioblastomas.[133] Recently, silencing of the VHL gene expression by DNA methylation, a novel mechanism of tumor suppressor gene inactivation, has been demonstrated in renal carcinoma.[134]

Multiple Tumor Suppressor 1 Gene (MST1, p16, CDKN2)

Recent deletion studies have demonstrated that loss of heterozygosity and/or homozygous deletion on chromosome 9p21-22 are common features for a number of tumor types, e.g., melanoma,[135-137] esophageal cancer,[138] pancreatic adenocarcinoma,[139] nasopharyngeal cancer,[140] lung cancer,[135,137,141-143] bladder carcinoma,[144-147] renal cell carcinoma,[148] brain tumors,[137,149-151] leukemia,[137] and mesothelioma.[152] These results strongly suggest that chromosome 9p21-22 may harbor a tumor suppressor gene or genes. Using nearly 100 melanoma cell lines for homozygous deletion (physical) mapping, Weaver-Feldhaus et al[153] have localized a putative tumor suppressor gene, Multiple Tumor Suppressor 1 (MTS1), to a region of less than 40 kb on chromosome 9p21.[153] Cloning of this gene reveals that the MTS1 gene encodes p16, a previously identified inhibitor of cyclin-dependent kinase 4 (Cdk4I).[154,155] Active cyclin D-Cdk4 complexes phosphorylate Rb protein and release the transcriptional factor E2F from the Rb-E2F complex *(Fig.1)*. Free and active E2F triggers G1 to S phase transition of the cell cycle *(Fig.1)*. p16 binds to and inactivates Cdk4 and therefore arrests the cell cycle in G1 phase *(Fig.1 and 2)*. Mutations of the MTS1 gene have been found in hereditary, sporadic as well as cultured melanomas,[152,154,156] primary brain cancer,[128] esophageal cancer,[159,160] bladder cancer,[145,161] pancreatic cancer,[139] and head and neck cancer.[145] Furthermore, the absence of p16(INK4) protein has been found to be restricted to the subset of lung cancer lines that retains wild-type RB.[162] However, the induction of the MTS1 gene has not yet been elucidated.

Other CDK inhibitors, e.g., p15,[163-165] p18,[17] p21,[43,44,166-168] p27,[169-172] p28,[173] may also be potential tumor suppressor genes, since they are negative regulators for the cell cycle. This question can only be answered by deletion and mutation studies of these genes. We investigated the possible involvement of the WAF1/CIP1 gene in prostate cancer. Our preliminary results suggest that WAF1/CIP1 gene is mutated in some prostate cancer patients (X Gao and KV Honn, unpublished observations).

BRCA1 Gene

A genetic linkage study mapped one form of breast cancer susceptibility to the BRCA1 locus on chromosome 17q21.[174] Subsequent investigations support the linkage and physically mapped the BRCA1 gene to a small region of 17q21 in breast-ovarian cancer families[175-181] (see 182 for a discussion). LOH studies also suggested that tumor suppressor gene(s) in chromosome 17q12-21 is important for the development of both familial and sporadic breast and ovarian cancer[183] (see 176, 184 for review). Recent positional cloning of the BRCA1 gene from that region allows mutational analysis of the BRCA1 gene in both inherited and sporadic forms of breast cancer.[8] Both germline and somatic BRCA1 mutations have been found in breast-ovarian cancer families, supporting the concept that the BRCA1 gene is indeed the tumor suppressor gene for familial breast-ovarian cancer.[8,178,181,185-187] The BRCA1 gene, containing 22 coding exons, spans approximately 100 kb of genomic DNA.[8] The full-length cDNA is 7.8 kb in size and encodes a protein product of 1863 amino acids.

The gene is expressed in a tissue-specific pattern, with the highest expression in testis and thymus and detectable levels in breast and ovary. The BRCA1 protein has a C3HC4 zinc-finger domain near the N terminus suggesting that BRCA1 may be able to bind to DNA and function as a transcription factor.[8] In fact, two substitutions affecting one of the last two cysteine residues in the zinc-finger were found in the breast cancer families indicating that at least some of the BRCA1 mutations may result in functional inactivation of the protein.[185-187]

Although the 17q21 LOH rate is relatively high (around 50%) in sporadic breast-ovarian cancer, the BRCA1 mutation is very uncommon.[188] When 32 breast and 12 ovarian cancers showing LOH were examined for possible BRCA1 mutations, only 4 germline (and no somatic) mutations were detected.[188] Out of 4 cases where the mutations were found, all patients had the onset between 24–44 years of age and two patients were identified retrospectively to have familial history. Collectively, the fact that LOH, but not mutation, of the BRCA1 locus occurs frequently in sporadic breast and ovarian cancers suggests that other gene(s) on 17q may be involved.[188] In fact, there are three common deletion regions other than the BRCA1 locus identified in sporadic breast cancer[189] (see 183 for a review).

Furthermore, the genetic heterogeneity of familial breast cancer has been documented by a new linkage analysis demonstrating a second breast cancer gene (BRCA2) on chromosome 13q12-13 which may account for as many familial breast cancer cases as the BRCA1 gene,[190] and complicated by the fact that mutations of the ataxia telangiectasia gene (BRCA3?) may cause breast cancer predisposition.[31,191] In contrast familial male breast cancer is not linked to the BRCA1 on chromosome 17q21.[192]

Prostate cancer was found to be the most common malignancy after breast cancer in the breast and ovarian cancer families segregated with chromosome 17q.[180,193] This suggests that gene(s) on 17q also may be involved in prostate cancer, in addition to breast and ovarian cancers. In an LOH study of 5 loci on 17q in 23 patients with primary prostatic adenocarcinoma, we observed LOH of at least one 17q locus in 11 of the 21 informative cases (52%) analyzed.[182] A commonly deleted region covering the D17S855 locus (intragenic to the BRCA1 gene) was found. Our data suggest that the BRCA1 and/or other gene(s) on 17q21 may be important in the pathogenesis of prostate cancer.[182] Finer deletion mapping of this region is currently underway.

Chromosomal Regions Harboring Potential Recessive Oncogenes

Other unidentified tumor suppressor genes have been suggested by LOH studies and gene transfer experiments. To date, several other tumor suppressor loci have been identified through LOH, e.g., 3p14-12 for renal cell carcinoma (194), 3p24-ter, 3p21.3 and 3p14-cen[195] and 13q14.3 (telomeric to the RB gene;)[196] for head and neck cancer, 5q34-ter for non-small cell lung cancer.[197] 9q22.3-31 (distal to the p16 gene) for basal cell carcinoma,[198] 11q22-24 for cervical carcinoma,[199] 1p32-pter,[200] 11q13,[201] 11q22-23.3[202] and 16q24.2-pter[203] for breast cancer, 19q for gliomas.[204]

Carter et al[25] first demonstrated frequent LOH of 10p and 16q in prostate cancer. Subsequent studies supported this finding and documented LOH of an additional chromosome region, i.e., 10q and 5q.[24,205,206] We have demonstrated that 32% of the primary prostate cancers showed LOH at microsatellite loci spanning chromosome 6p12-24 suggesting that this region may harbor a tumor suppressor gene or genes.[207] One of the potential tumor suppressor genes is the p21/WAF1/CIP1 gene, which is a general inhibitor for CDKs and is inducible by wt-p53.[43,44,52,208] A number of groups have demonstrated frequent allelic deletion on chromosome 8p in prostate cancer, suggesting that chromosome 8p12-21 and 8p22 may contain tumor suppressor genes.[209-212] In fact, by using 29 cloned and mapped fragments of yeast artificial chromosomes (YACs) selected for the 13 megabase segment of 8p22 as probes for homozygous deletion mapping, Bova et al. have narrowed the common deletion region to an approximately 1 megabase stretch centered around the D8S549 locus.[213] Finally, tumor suppressor genes may also reside in chromosomes 7q31.1-31.2 and 11p11.2-p13.[214-216]

Conclusions

Cancer is thought to arise from the accumulation of several genetic mutations which include both the mutations that activate dominant oncogenes and the mutations that inactivate recessive oncogenes or tumor suppressor genes. p53 is one of the most intensively studied tumor suppressor genes. Its product, the p53 protein cannot only serve as a true transactivator for targeted gene transcription but also inhibits DNA replication, mediating the arrest of the cell cycle at the G1 phase, thus serves as a guardian of the genome protecting it from damage. Inactivation of the p53 protein by mutation, or by its interactions with amplified cellular p53-associating MDM2 protein or with viral proteins, can result in malignancy. Rb, works at a different level of the same signaling pathway as p53 and also can mediate cell cycle G1 arrest. Cyclin-dependent kinase inhibitors (e.g., $p16^{MTS1}$ and $p21^{WAF1/CIP1}$) also could serve as mediators to stop the cell cycle. While the function of those tumor suppressor genes is known, the exact mechanisms of action for other tumor suppressor genes, such as DCC, APC, MCC, VHL and BRCA1 genes, are largely unelucidated. As our understanding of cell cycle regulation by tumor supressor genes increases, molecular and cellular assays may become available to assess the cell cycle controls defective

in specific cancers. Such characterization may not only dictate the choice and schedule of the currently existing phase- or cell cycle-specific agents to be used in cancer therapy, but also lead to an explosion of novel mechanistic targets for chemotherapeutic intervention.

Acknowledgement

We gratefully acknowledge Dr. Bert Vogelstein (John Hopkins University) for communicating unpublished data, Dr. Eric R. Fearon (Yale University) for providing rabbit antibodies against DCC, and Mr. Alex Zacharek, Ms. Ning Wu, and Ms. Alicia Salkowski for excellent technical assistance. The work in our laboratory is supported by Harper Hospital Development Fund (A. T. P. and K. V. H.), Detroit Medical Center, Wayne State University.

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