

TUMOR-SUPPRESSOR GENES

Molecular Oncology 2012

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TUMOR-SUPPRESSOR GENES - Lecture Outline

1. Summary of tumor suppressor genes
2. P53
3. Rb
4. BRCA1 and 2
5. APC and DCC
6. PTEN and PPA2
7. LKB1
8. P16
9. WT1 and WTX
10. Epigenetic changes
11. miRNAs

TUMOR-SUPPRESSOR GENES -Introduction

Fusion of tumor cells with normal cells has been found to result in a loss of transformed properties. This suggests there are tumor suppressing activities in normal cells. Further support for this concept is provided by chromosomal deletions associated with some malignancies. The following is a list of tumor suppressor genes. Note there can be hereditary and sporadic defects for these genes.

<u>Gene</u>	<u>Cancer type</u>	<u>Hereditary syndrome</u>
APC	Colon cancer	Familial adenomatous polyposis
BRCA1	Breast cancer	
BRCA2	Breast cancer	
DCC	Colon cancer	
NF1	Neurofibromas	Neurofibromatosis type 1
NF2	Schwannomas and Meningiomas	Neurofibromatosis type 2
p53	Many types	Li-Fraumeni syndrome
PTEN	Gliomas	
Rb	Retinoblastoma	Retinoblastoma
VHL	Kidney and other tumors	von Hippel-Lindau syndrome
WT1	Wilms tumor	Wilms tumor

In hereditary nonpolyposis colorectal cancer (HNPCC) defects have been noted in two genes coding for proteins used in DNA repair, namely MSH2 and MLH1. With defects in these genes there will be a high mutation frequency.

Reference slide

Table 7.1 Human tumor suppressor genes that have been cloned

Name of gene	Chromosomal location	Familial cancer syndrome	Sporadic cancer	Function of protein
<i>RUNX3</i>	1p36	—	gastric carcinoma	TF co-factor
<i>HRPT2</i>	1q25–32	parathyroid tumors, jaw fibromas	parathyroid tumors	chromatin protein
<i>FH</i>	1q42.3	familial leiomyomatosis ^a	—	fumarate hydratase
<i>FHIT</i>	3p14.2	—	many types	diadenosine triphosphate hydrolase
<i>RASSF1A</i>	3p21.3	—	many types	multiple functions
<i>TGFBR2</i>	3p2.2	HNPCC	colon, gastric, pancreatic carcinomas	TGF- β receptor
<i>VHL</i>	3p25	von Hippel–Lindau syndrome	renal cell carcinoma	ubiquitylation of HIF
<i>hCDC4</i>	4q32	—	endometrial carcinoma	ubiquitin ligase
<i>APC</i>	5p21	familial adenomatous polyposis coli	colorectal, pancreatic, and stomach carcinomas; prostate carcinoma	β -catenin degradation
<i>NKX3.1</i>	8p21	—	prostate carcinoma	homeobox TF
<i>p16^{INK4A}</i> ^b	9p21	familial melanoma	many types	CDK inhibitor
<i>p14^{ARF}</i> ^c	9p21	—	all types	p53 stabilizer
<i>PTC</i>	9q22.3	nevoid basal cell carcinoma syndrome	medulloblastomas	receptor for hedgehog GF
<i>TSC1</i>	9q34	tuberous sclerosis	—	inhibitor of mTOR ^f
<i>BMPR1</i>	10q21–22	juvenile polyposis	—	BMP receptor
<i>PTEN</i> ^d	10q23.3	Cowden's disease, breast and gastrointestinal carcinomas	glioblastoma; prostate, breast, and thyroid carcinomas	PIP ₃ phosphatase
<i>WT1</i>	11p13	Wilms tumor	Wilms tumor	TF
<i>MEN1</i>	11p13	multiple endocrine neoplasia	—	histone modification, transcriptional repressor

^aFamilial leiomyomatosis includes multiple fibroids, cutaneous leiomyomas, and renal cell carcinoma. The gene product is a component of the tricarboxylic cycle.

^bAlso known as *MTS1*, *CDKN2*, and *p16*.

^cThe human homolog of the murine *p19^{ARF}* gene.

^dAlso called *MMAC* or *TEP1*.

^e*SDHS* encodes the succinate–ubiquinone oxidoreductase subunit D, a component of the mitochondrial respiratory chain complex II.

^fmTOR is a serine/threonine kinase that controls, among other processes, the rate of translation and activation of Akt/PKB. TSC1 (hamartin) and TSC2 (tuberin) control both cell size and cell proliferation.

^gThe *CBP* gene is involved in chromosomal translocations associated with AML. These translocations may reveal a role of a segment of CBP as an oncogene rather than a tumor suppressor gene.

^hAlso termed Carney complex.

ⁱEncodes the Smad4 TF associated with TGF- β signaling; also known as *MADH4* and *SMAD4*.

^jThe human SNF5 protein is a component of the large Swi/Snf complex that is responsible for remodeling chromatin in a way that leads to transcriptional repression through the actions of histone deacetylases. The rhabdoid predisposition syndrome involves susceptibility to atypical teratoid/rhabdoid tumors, choroid plexus carcinomas, medulloblastomas, and extra-renal rhabdoid tumors.

Adapted in part from E.R. Fearon, *Science* 278:1043–1050, 1997; and in part from D.J. Marsh and R.T. Zori, *Cancer Lett.* 181:125–164, 2002.

Table 7-1 part 1 of 2 The Biology of Cancer (© Garland Science 2007)

Reference slide

Table 7.1 Human tumor suppressor genes that have been cloned

Name of gene	Chromosomal location	Familial cancer syndrome	Sporadic cancer	Function of protein
<i>BWS/CDKN1C</i>	11p15.5	Beckwith–Wiedemann syndrome	—	p57 ^{Kip2} CDK inhibitor
<i>SDHD</i>	11q23	familial paraganglioma	pheochromocytoma	mitochondrial protein ^e
<i>RB</i>	13q14	retinoblastoma, osteosarcoma	retinoblastoma; sarcomas; bladder, breast, esophageal, and lung carcinomas	transcriptional repression; control of E2Fs
<i>TSC2</i>	16p13	tuberous sclerosis	—	inhibitor of mTOR ^f
<i>CBP</i>	16p13.3	Rubinstein–Taybi	AML ^g	TF co-activator
<i>CYLD</i>	16q12–13	cylindromatosis	—	deubiquitinating enzyme
<i>CDH1</i>	16q22.1	familial gastric carcinoma	invasive cancers	cell–cell adhesion
<i>BHD</i>	17p11.2	Birt–Hogg–Dube syndrome	kidney carcinomas, hamartomas	unknown
<i>TP53</i>	17p13.1	Li–Fraumeni syndrome	many types	TF
<i>NF1</i>	17q11.2	neurofibromatosis type 1	colon carcinoma, astrocytoma	Ras–GAP
<i>BECN1</i>	17q21.3	—	breast, ovarian, prostate	autophagy
<i>PRKAR1A</i>	17.q22–24	multiple endocrine neoplasia ^h	multiple endocrine tumors	subunit of PKA
<i>DPC4ⁱ</i>	18q21.1	juvenile polyposis	pancreatic and colon carcinomas	TGF-β TF
<i>LKB1/STK11</i>	19p13.3	Peutz–Jegher syndrome	hamartomatous colonic polyps	serine/threonine kinase
<i>RUNX1</i>	21q22.12	familial platelet disorder	AML	TF
<i>SNF5^j</i>	22q11.2	rhabdoid predisposition syndrome	malignant rhabdoid tumors	chromosome remodeling
<i>NF2</i>	22q12.2	neurofibroma-position syndrome	schwannoma, meningioma; ependymoma	cytoskeleton–membrane linkage

^aFamilial leiomyomatosis includes multiple fibroids, cutaneous leiomyomas, and renal cell carcinoma. The gene product is a component of the tricarboxylic cycle.

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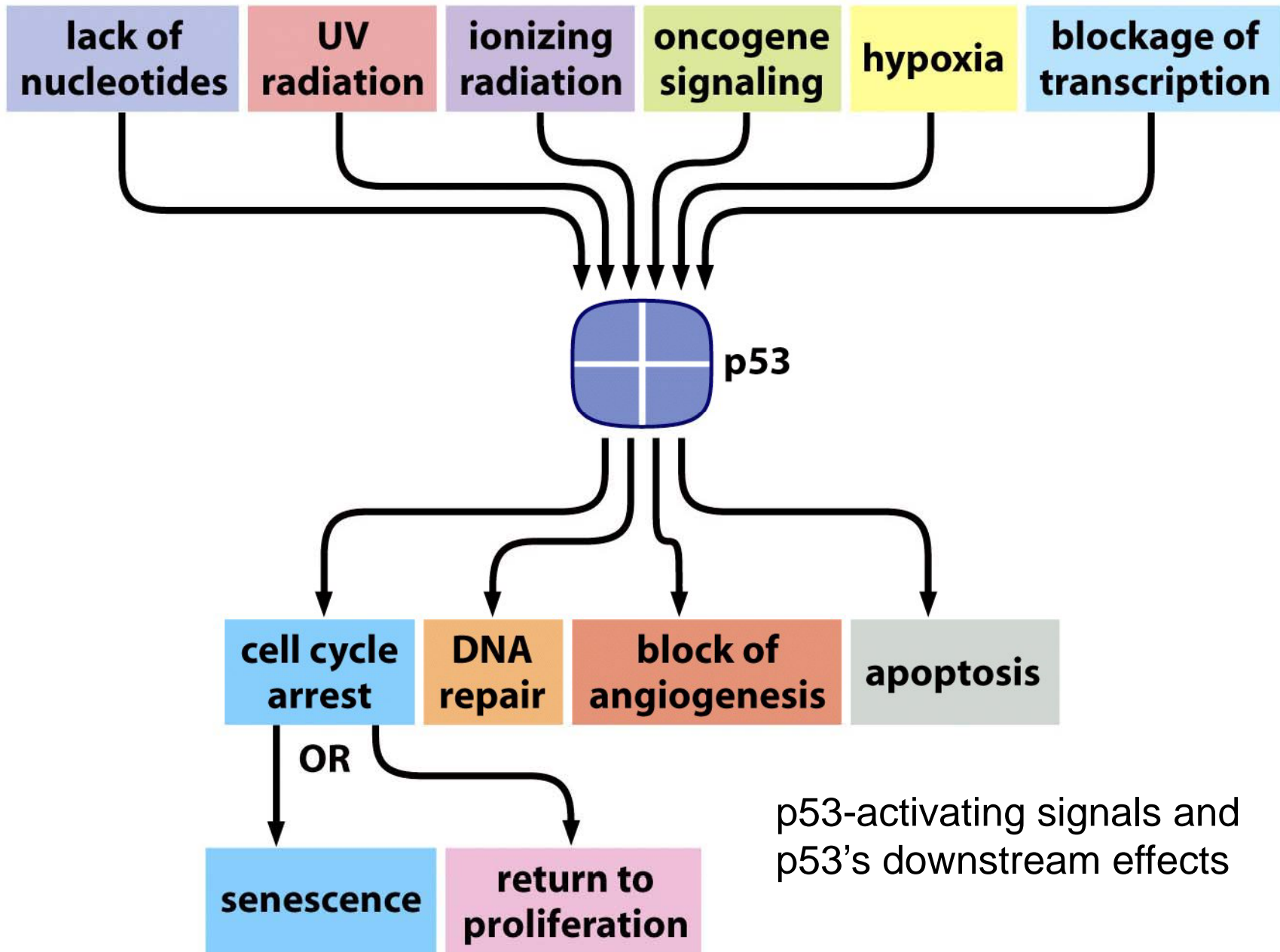
p53

Mutations in the p53 gene are found in a greater percentage of tumors than any other gene mutation. The situation with the p53 gene is complicated by the fact that mutation can result in

1. the loss of tumor suppressor function
- 2 oncogene activity including a dominant negative effect which overrides the influence of the wild type gene.

Hot spots have been identified in the p53 gene which are prone to mutation. Exposure to aflatoxin B1 causes a G->T transversion at codon 249 which is not generally seen in geographical regions with low exposure to aflatoxin. In the Li-Fraumeni syndrome, there is a germ-line mutation of the p53 gene resulting in a high incidence of cancer particularly tumors of the adrenal cortex, breast and brain and osteosarcomas.

The p53 gene takes its name from the size of the 53 kd gene product. There are phosphorylation sites on the p53 protein including one which is phosphorylated by a cyclin-dependent kinase and which may be associated with cell cycle dependent translocation into the cell nucleus.



p53-activating signals and p53's downstream effects

Figure 9-8 The Biology of Cancer (© Garland Science 2007)

p53

The p53 protein is a transcriptional regulator that has been associated with blocking cell cycle progression and inducing apoptosis in some systems. These effects may be mediated by the products of genes whose expression is enhanced by the p53 protein including the p21^{WAF1/Cip1} gene and the Bax gene. The p21^{WAF1/Cip1} is known to be an inhibitor of cyclin-dependent kinase activity and can block cell cycle progression. The Bax protein is a promoter of apoptosis. The p53 gene is activated by DNA damage. It is thought to be important in normal cells to slow the cell cycle when DNA is damaged to permit DNA repair before the DNA is replicated. Failing this it may be preferable for the cell to die rather than perpetuate a damaged genome. Some of the action of the p53 gene on DNA repair may be mediated by activation of the Growth Arrest DNA damage gene, GADD45.

the function of the p53 protein can be inhibited by binding to the product of the mdm-2 gene. This may constitute part of a feedback loop because the mdm-2 gene is activated by the p53 protein. When the mdm-2 gene is overexpressed as in some sarcomas it serves as an oncogene by suppressing the function of the p53 protein.

Post-translational modification of p53

The p53 protein is subject to a variety of post-translational modifications.

Phosphorylation and acetylation of p53 generally results in its stabilization and accumulation in the nucleus, followed by activation. Several protein kinases can phosphorylate p53.

Mutant p53 is generally phosphorylated and acetylated at sites that are known to stabilize wild type p53 and could cause accumulation of dysfunctional p53 functioning as an oncogene.

Overexpression of MDM2 E3 ubiquitin ligase results in the deactivation of p53 in many tumors.

Reference: A.M. Bode and Z. Dong. Post-translational modification of p53 in tumorigenesis. *Nature Reviews Cancer* 4: 793-803, 2004.

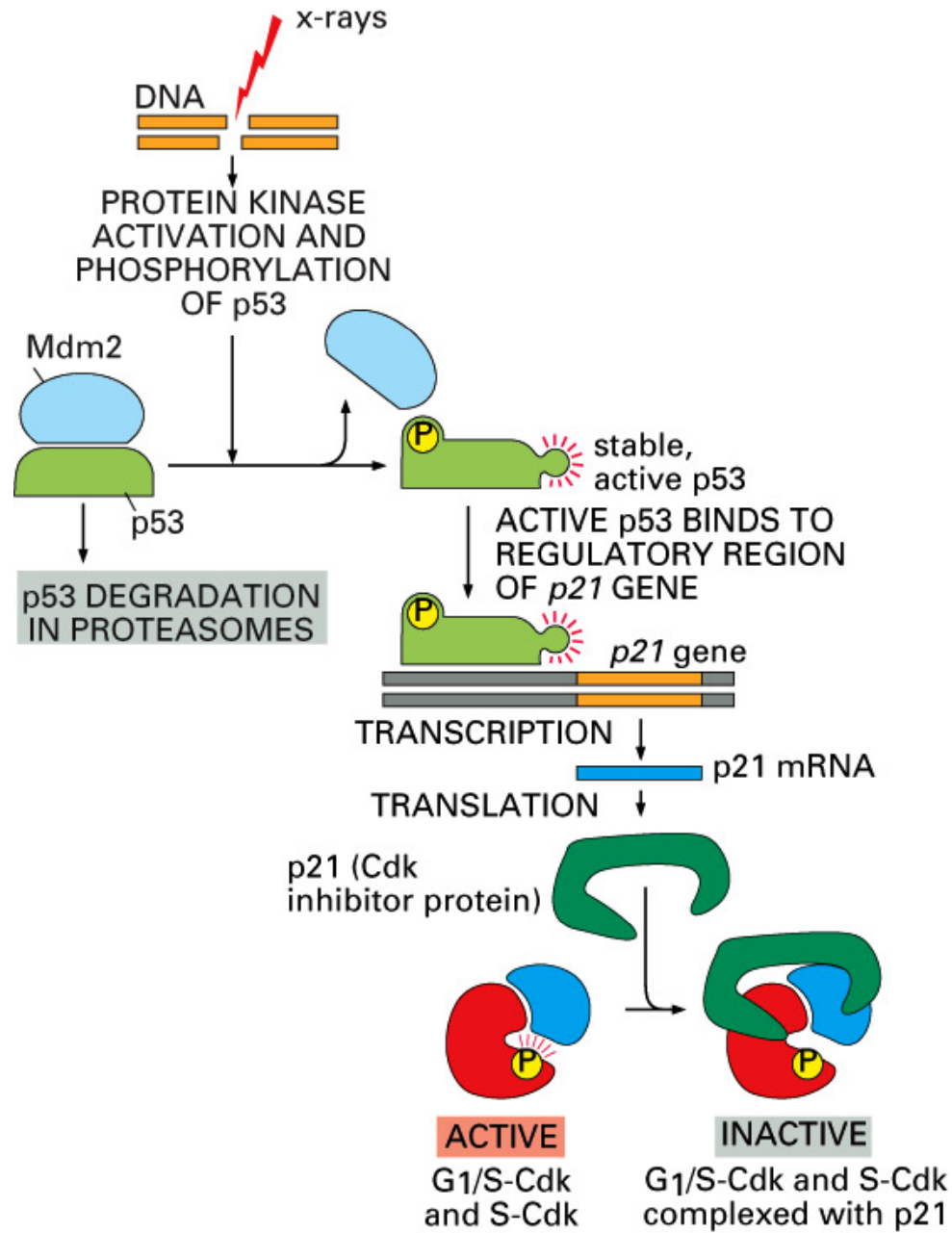


Figure 17-33. Molecular Biology of the Cell, 4th Edition.

Rb

Retinoblastoma is an eye tumor of young children that occurs in a hereditary or a sporadic form. Deletions have been found in chromosome 13 associated with retinoblastoma. Inheritance of one defective gene puts the individual at greater risk. A somatic mutation in the other **Rb** gene will cause cancer whereas somatic mutations in two genes would be required in the normal individual.

The Rb gene codes for a 105 kd protein. When hypophosphorylated p105 Rb exerts a growth restraining influence in the G1 phase of the cell cycle. Phosphorylation of the Rb protein inhibits its growth regulatory action. The Rb protein is a substrate for phosphorylation by cyclin-dependent kinases. Hyperphosphorylated Rb protein binds less tightly to the nucleus and less tightly to the E2F transcription factor which activates some genes for cell cycle progression. In the normal cell cycle, Rb becomes hyperphosphorylated at the G1/S transition and is released from the E2F transcription factor. The Rb protein can also bind specific DNA sequences and serve as a transcriptional regulator.

Some transforming DNA viruses encode proteins that can bind with the Rb protein and block its function. These viral proteins include adenovirus E1A protein, SV40 large T antigen, E7 protein of human papilloma viruses 16 and 18 and polyoma middle T antigen.

The Rb gene is required for normal development. Knockout mice die at about 14 to 15 days of embryonic development.

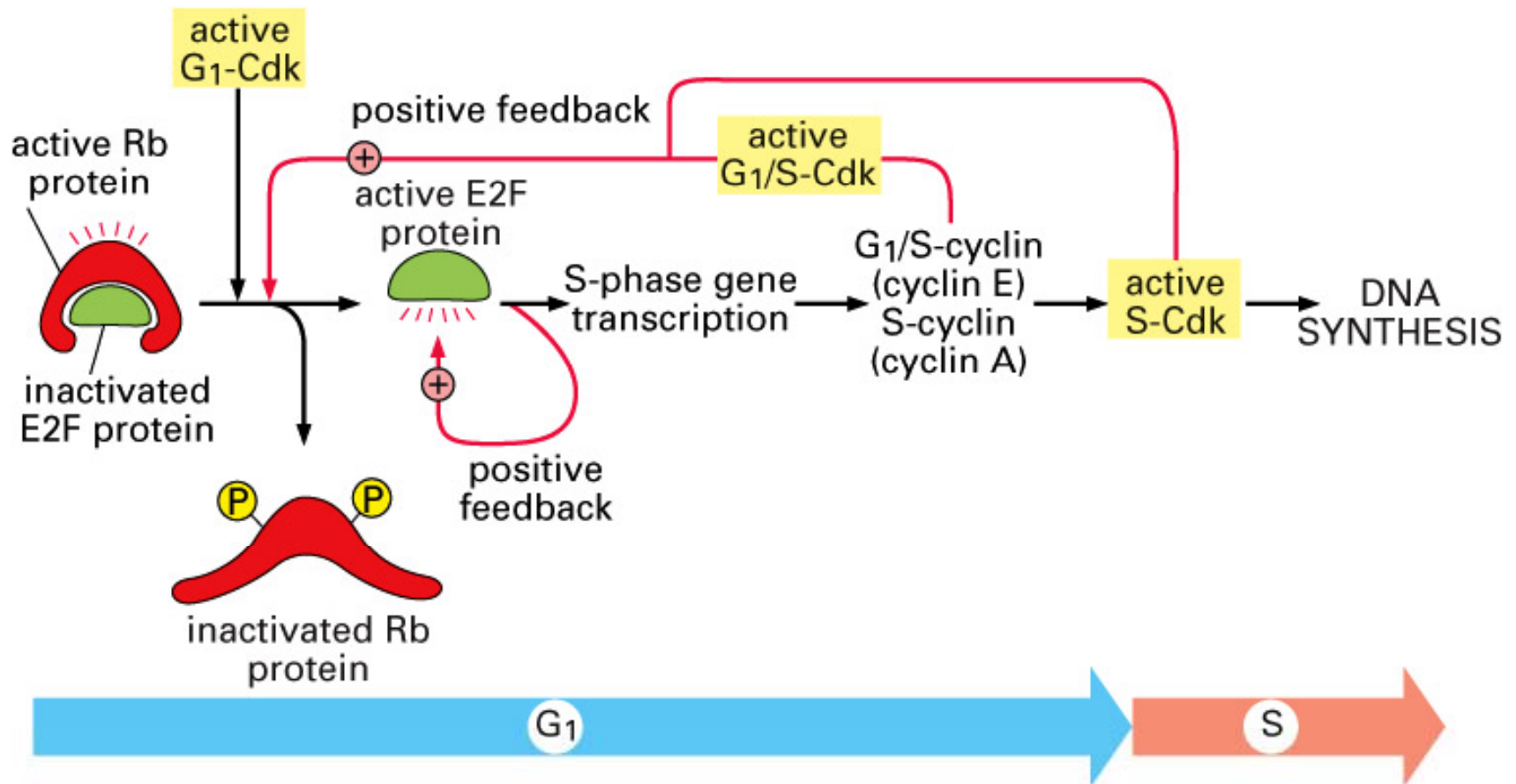


Figure 17-30. Molecular Biology of the Cell, 4th Edition.

BRCA1 and BRCA2 GENES

Mutations in the BRCA1 and BRCA2 genes impart increased susceptibility to breast cancer. Most cases are sporadic but some cases are familial. The BRCA1 gene codes for a large nuclear phosphoprotein whose expression and phosphorylation is cell cycle dependent. It is probably a DNA-binding transcription factor and also involved in DNA repair. Although BRCA1 has a ubiquitin ligase domain, this does not appear to be critical for its tumor suppressor function, whereas the BRCT domain appears essential and recognizes a phosphorylated serine consensus sequence.

Mutations in the BRCA2 tumor-suppressor gene cause genomic instability and predisposition to cancer.

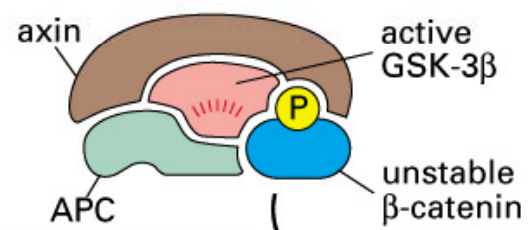
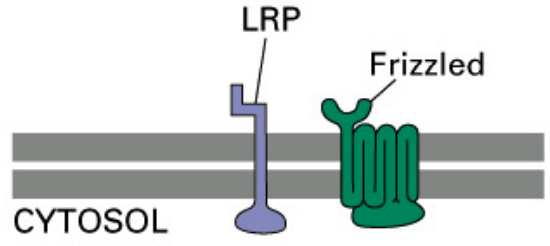
BRCA2 appears to be required to prevent the breakdown of stalled replication forks. Disruption of this function leads to chromosomal rearrangements that occur spontaneously in dividing cells that have mutations in BRCA2.

APC

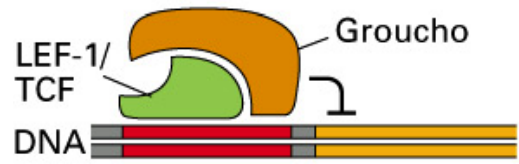
In familial adenomatous polyposis (FAP) the colon is normal at birth, but during the first 20 years of life, hundreds of small polyps appear in the colon. The polyps are asymptomatic but there is a risk of progression to colon cancer that approaches 100% by age 50.

The gene responsible is APC (adenomatous polyposis coli) which is involved in the degradation of beta-catenin.

(A) **WITHOUT Wnt SIGNAL**



PHOSPHORYLATED β -CATENIN IS UBIQUITYLATED AND DEGRADED IN PROTEASOME



Wnt-RESPONSIVE GENES OFF

Figure 15-72 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

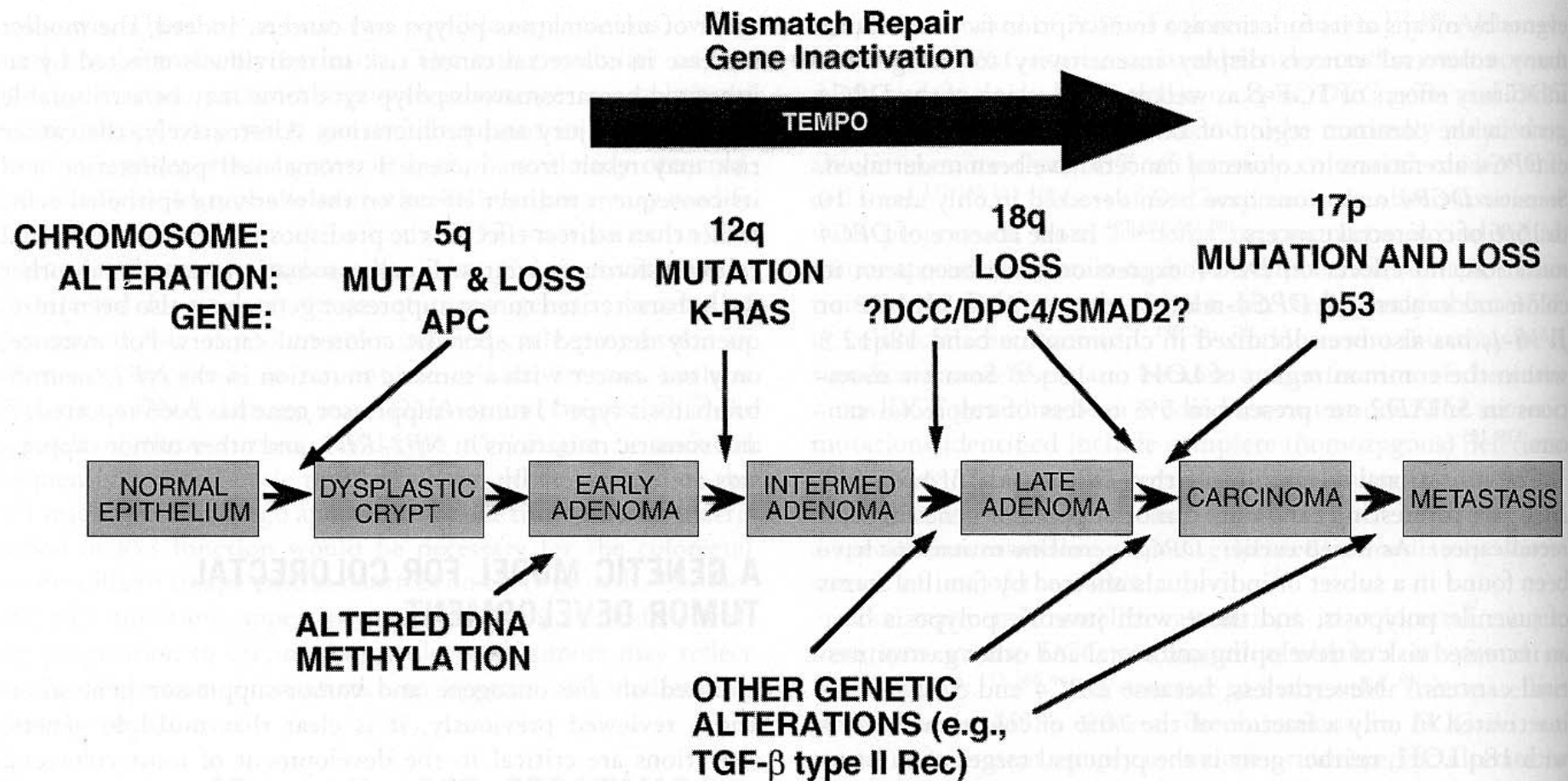


FIGURE 15–12. Genetic model of colorectal cancer. The majority of colorectal cancers are believed to arise from adenomatous polyps over a period of years or even decades. The inherited and somatic genetic alterations believed to underlie tumor initiation and progression are indicated and are discussed in detail in the text. Although their order is not invariant, the mutations show strong association with particular stages of tumorigenesis. In about 15% of colorectal cancers, germline and somatic mutations as well as epigenetic mechanisms inactivate mismatch-repair gene function. Cells with mismatch-repair gene inactivation manifest a mutator phenotype, and the mutation rate and tempo of tumor progression are clearly altered. Mutations inactivating the transforming growth factor- β type II receptor (TGF- β type II Rec) appear to be restricted to those tumors with mismatch-repair gene inactivation (i.e., tumors with the MSI⁺ phenotype). (Modified from Fearon ER, Vogelstein B: A genetic model for colorectal tumorigenesis. *Cell* 61:759, 1990.)

Reference slide

Table 7.2 Examples of hypermethylated genes found in human tumor cell genomes

Name of gene	Nature of protein function	Type of tumor
<i>RARβ2</i>	nuclear receptor for differentiation	breast, lung
<i>p57^{Kip2}</i>	CDK inhibitor	gastric, pancreatic, hepatic; AML
<i>TIMP3</i>	inhibitor of metalloproteinases	diverse tumors
<i>IGFBP</i>	sequesters IGF-1 factor	diverse tumors
<i>CDKN2A/p16^{INK4A}</i>	inhibitor of CDK4/6	diverse tumors
<i>CDKN2B/p15^{INK4B}</i>	inhibitor of CDK4/6	diverse tumors
<i>p14^{ARF}</i>	inhibitor of HDM2/MDM2	colon, lymphoma
<i>APC</i>	inducer of β-catenin degradation	colon carcinomas
<i>p73</i>	aids p53 to trigger apoptosis	diverse tumors
<i>GSTP1</i>	mutagen inactivator	breast, liver, prostate
<i>MGMT</i>	DNA repair enzyme	colorectal
<i>CDH1</i>	cell-cell adhesion receptor	bladder, breast, colon, gastric
<i>DAPK</i>	kinase involved in cell death	bladder
<i>MLH1</i>	DNA mismatch repair enzyme	colon, endometrial, gastric
<i>TGFBR2</i>	TGF-β receptor	colon, gastric, small-cell lung
<i>THBS1</i>	angiogenesis inhibitor	colon, glioblastoma
<i>RB</i>	cell-cycle regulator	retinoblastoma
<i>CASP8</i>	apoptotic caspase	neuroblastoma, SCLC
<i>APAF1</i>	pro-apoptotic cascade	melanoma
<i>CTMP</i>	inhibitor of Akt/PKB	glioblastoma multiforme

Adapted in part from C.A. Eads et al., *Cancer Res.* 61:3410–3418, 2001.

PTEN (Phosphatase and Tensin homolog deleted on chromosome Ten)

The PTEN gene is frequently mutated in human cancer, particularly gliomas. The PTEN protein can dephosphorylate phosphatidyl inositol 3,4,5 trisphosphate and thereby antagonize the phosphatidylinositol-3-kinase signaling pathway. PTEN negatively regulates intracellular levels of phosphatidylinositol-3,4,5-trisphosphate in cells and functions as a tumor suppressor by negatively regulating AKT/PKB signaling pathway.

PTEN may also inhibit cell migration through protein phosphatase activity on a threonine phosphate residue.

Reference: Raftopoulou et al. Science 303, 1179-1181 (2004).

PP2A

PP2A is a serine/threonine phosphatase consisting in vivo of 3 subunits. The catalytic subunit (C-subunit) is present in 2 isoforms, a and b, which show the highest evolutionary conservation of all known enzymes, supporting the idea that they serve crucial functions. The catalytic subunit is constitutively associated with a structural/regulatory subunit (A-subunit), which exists in 2 isoforms encoded by different genes. The A-subunit is indispensable for the interaction of the catalytic subunit with the third regulatory subunit (B-subunit).

Mutations in PPP2R1B, the gene encoding the beta-isoform of the A-subunit of PP2, have been recently described. The gene is localized on human chromosome 11q23, a region undergoing LOH in several tumors, including colon and lung cancer.

Reference: Sloan-Kettering Institute > Cancer Biology & Genetics > Pier Paolo Pandolfi > Projects > Phosphatases and Cancer

PP2A

Protein Phosphatase 2A (PP2A) plays a role in the critical cellular processes of protein synthesis, DNA replication, transcription, and metabolism. Small t antigen of SV40 interacts with the PP2A. This interaction reduces the ability of PP2A to inactivate ERK1 and MEK1 protein kinases, resulting in stimulation of proliferation of cells.

LKB1

Metformin and reduced risk of cancer in diabetic patients

Metformin, widely given to patients with type 2 diabetes, works by targeting the enzyme AMPK (AMP activated protein kinase), which induces muscles to take up glucose from the blood. A recent breakthrough has found the upstream regulator of AMPK to be a protein kinase known as LKB1. LKB1 is a well recognized tumor suppressor. The Peutz-Jeghers tumor-suppressor gene encodes a protein-threonine kinase, LKB1, that phosphorylates and activates AMPK. Activation of AMPK by metformin and exercise requires LKB1, and this may also explain why exercise is beneficial in the primary and secondary prevention of certain cancers. Metformin use in patients with type 2 diabetes may reduce their risk of cancer.

J.M.M. Evans, L.A. Donnelly, A.M. Emslie-Smith, D.R. Alessi and A.D. Morris. *Brit. Med. J.* 330:1304-1305 (2005),

R.J. Shaw et al., *Science* 310: 1642-1646, 2005.

J.R. Fay, V. Steele and J.A. Crowell. *Cancer Prev. Res.* 2: 301-309, 2009.

p16

The *INK4a/ARF* locus is of critical importance in tumor suppression. This locus is inactivated in about 40% of human cancers, a frequency only comparable with that of p53 inactivation. The *INK4a/ARF* locus encodes two tumor suppressors, p16^{INK4a} and p14^{ARF}/p19^{ARF} (p14 when referred to the human protein and p19 when referred to the mouse protein), which share exons 2 and 3 but differ in their first exons and their respective promoters.

Protein p16^{INK4a} inhibits the activity of the CDK4,6/cycD kinases, thus contributing to the maintenance of the active, growth suppressive form of the retinoblastoma family of proteins.

Matheu, A., Klatt, P., and Serrano, M. Regulation of the *INK4a/ARF* locus by histone deacetylase inhibitors. *J. Biol. Chem.* 280, 42433-42441, 2005

p16

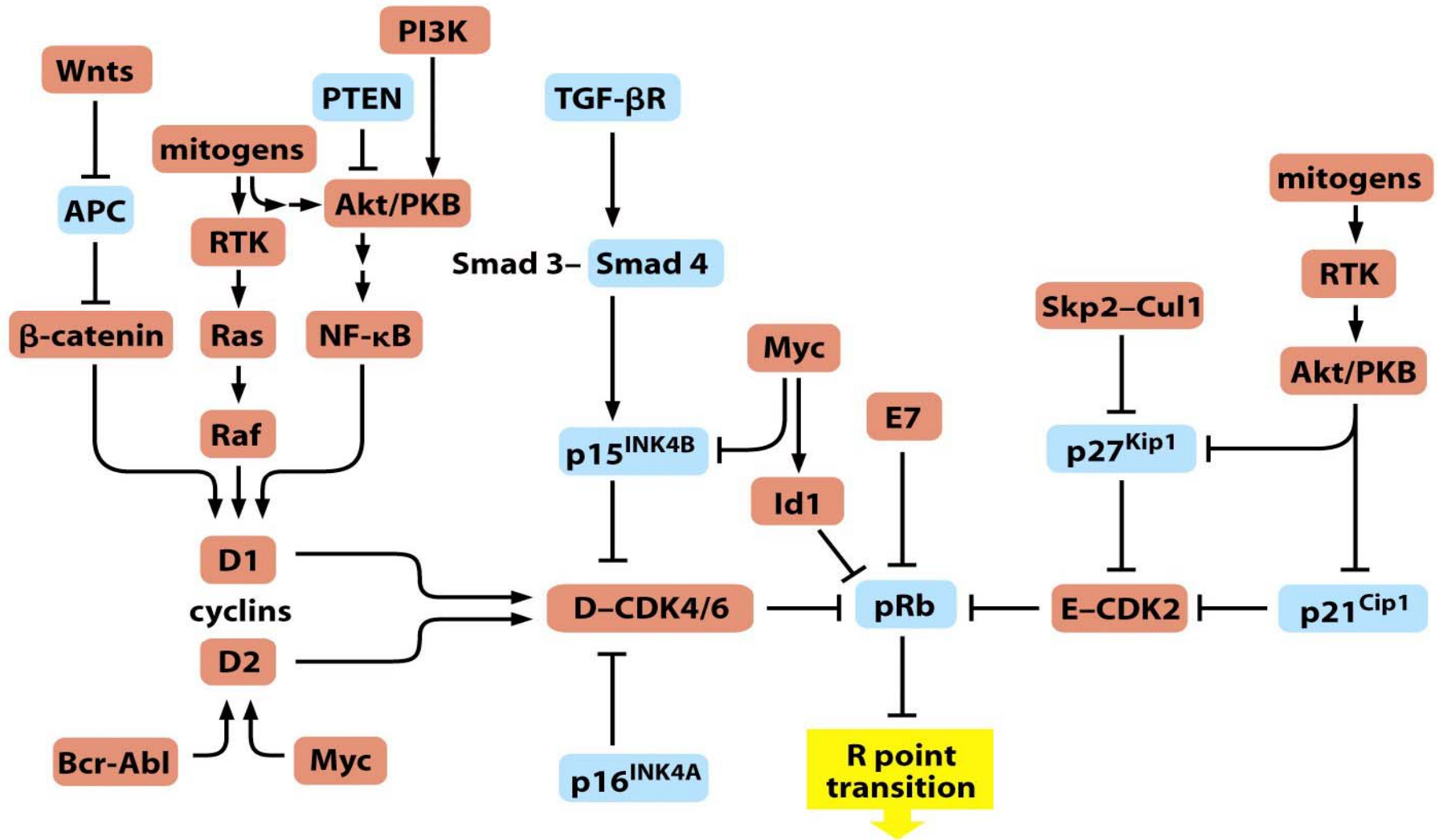


Figure 8-35 The Biology of Cancer (© Garland Science 2007)

An X Chromosome Gene, WTX, is Commonly Inactivated in Wilm's Tumor

Wilm's tumor is a pediatric kidney cancer associated with inactivation of the WT1 tumor suppressor gene in 5-10% of cases.

The WTX gene is inactivated in approximately one third of Wilm's tumors.

In contrast to the biallelic inactivation of autosomal tumor-suppressor genes, WTX is inactivated by a monoallelic "single-hit" event targeting the single X chromosome in tumors in males and the active X chromosome in tumors from females.

Reference: Rivera et al., Science 315: 642-645, 2007

Epigenetic Changes

Some of the mechanisms involved in carcinogenesis may be epigenetic rather than genetic changes. Epigenetic changes include methylation of DNA and side-chain modification of histones including methylation and acetylation.

One mechanism for the down regulation of tumor suppressor genes is methylation of promoter regions.

Many groups are studying the combined action of inhibitors of DNA methylation and inhibitors of histone deacetylases as potential chemotherapeutic regimens.

Several components of the SWI/SNF chromatin remodeling complex appear to have a tumor suppressor role.

miRNAs

The deletion of the let-7 miRNA gene in *C. elegans* cause an uncontrolled proliferation of stem cells and overexpression of the ras gene. Lung cancer patients in Japan with the lowest levels of let-7 expression were found to have the worst prognosis.

On the other hand, genes for some miRNAs may serve as oncogenes. A group of 13 miRNAs were reported to form a signature associated with prognosis and disease progression in patients with chronic lymphocytic leukemia (CLL).

mir21 is recognized as an oncomir that targets the expression of several tumor suppressor genes.

TUMOR-SUPPRESSOR GENES -Suggested reading

D. Cosgrove, B.H. Park and B. Vogelstein, In Holland-Frei Cancer Medicine 8th Edition, Part II, Section 1, 7. Tumor-Suppressor Genes, 2010.

Robert Weinberg, The Biology of Cancer, Chapters 7-9, Garland Press, 2007.