TUMOR-SUPPRESSOR GENES

Molecular Oncology 2011

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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.

Gene	Cancer type	Hereditary syndrome
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
RUNX3	1p36	—	gastric carcinoma	TF co-factor
HRPT2	1q25–32	parathyroid tumors, jaw fibromas	parathyroid tumors	chromatin protein
FH	1q42.3	familial leiomyomatosis[a]	—	fumarate hydratase
FHIT	3p14.2	—	many types	diadenosine triphosphate hydrolase
RASSF1A	3p21.3	—	many types	multiple functions
TGFBR2	3p2.2	HNPCC	colon, gastric, pancreatic carcinomas	TGF-β receptor
VHL	3p25	von Hippel–Lindau syndrome	renal cell carcinoma	ubiquitylation of HIF
hCDC4	4q32	—	endometrial carcinoma	ubiquitin ligase
APC	5p21	familial adenomatous polyposis coli	colorectal, pancreatic, and stomach carcinomas; prostate carcinoma	β-catenin degradation
NKX3.1	8p21	—	prostate carcinoma	homeobox TF
$p16^{INK4A}$ [b]	9p21	familial melanoma	many types	CDK inhibitor
$p14^{ARF}$ [c]	9p21	—	all types	p53 stabilizer
PTC	9q22.3	nevoid basal cell carcinoma syndrome	medulloblastomas	receptor for hedgehog GF
TSC1	9q34	tuberous sclerosis	—	inhibitor of mTOR[f]
BMPR1	10q21–22	juvenile polyposis	—	BMP receptor
PTEN[d]	10q23.3	Cowden's disease, breast and gastrointestinal carcinomas	glioblastoma; prostate, breast, and thyroid carcinomas	PIP_3 phosphatase
WT1	11p13	Wilms tumor	Wilms tumor	TF
MEN1	11p13	multiple endocrine neoplasia	—	histone modification, transcriptional repressor

[a]Familial leiomyomatosis includes multiple fibroids, cutaneous leiomyomas, and renal cell carcinoma. The gene product is a component of the tricarboxylic cycle.
[b]Also known as *MTS1, CDKN2,* and *p16.*
[c]The human homolog of the murine $p19^{ARF}$ gene.
[d]Also called *MMAC* or *TEP1.*
[e]*SDHS* encodes the succinate–ubiquinone oxidoreductase subunit D, a component of the mitochondrial respiratory chain complex II.
[f]mTOR 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.
[g]The *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.
[h]Also termed Carney complex.
[i]Encodes the Smad4 TF associated with TGF-β signaling; also known as *MADH4* and *SMAD4.*
[j]The 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 deactylases. 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
BWS/CDKN1C	11p15.5	Beckwith–Wiedemann syndrome	—	$p57^{Kip2}$ CDK inhibitor
SDHD	11q23	familial paraganglioma	pheochromocytoma	mitochondrial protein[e]
RB	13q14	retinoblastoma, osteosarcoma	retinoblastoma; sarcomas; bladder, breast, esophageal, and lung carcinomas	transcriptional repression; control of E2Fs
TSC2	16p13	tuberous sclerosis	—	inhibitor of mTOR[f]
CBP	16p13.3	Rubinstein–Taybi	AML[g]	TF co-activator
CYLD	16q12–13	cylindromatosis	—	deubiquitinating enzyme
CDH1	16q22.1	familial gastric carcinoma	invasive cancers	cell–cell adhesion
BHD	17p11.2	Birt–Hogg–Dube syndrome	kidney carcinomas, hamartomas	unknown
TP53	17p13.1	Li–Fraumeni syndrome	many types	TF
NF1	17q11.2	neurofibromatosis type 1	colon carcinoma, astrocytoma	Ras-GAP
BECN1	17q21.3	—	breast, ovarian, prostate	autophagy
PRKAR1A	17.q22–24	multiple endocrine neoplasia[h]	multiple endocrine tumors	subunit of PKA
DPC4[i]	18q21.1	juvenile polyposis	pancreatic and colon carcinomas	TGF-β TF
LKB1/STK11	19p13.3	Peutz–Jegher syndrome	hamartomatous colonic polyps	serine/threonine kinase
RUNX1	21q22.12	familial platelet disorder	AML	TF
SNF5[j]	22q11.2	rhabdoid predisposition syndrome	malignant rhabdoid tumors	chromosome remodeling
NF2	22q12.2	neurofibroma-position syndrome	schwannoma, meningioma; ependymoma	cytoskeleton–membrane linkage

[a]Familial leiomyomatosis includes multiple fibroids, cutaneous leiomyomas, and renal cell carcinoma. The gene product is a component of the tricarboxylic cycle.
[b]Also known as *MTS1, CDKN2,* and *p16.*
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[i]Encodes the Smad4 TF associated with TGF-β signaling; also known as *MADH4* and *SMAD4.*
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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 2 of 2 The Biology of Cancer (© Garland Science 2007)

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 overides 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.

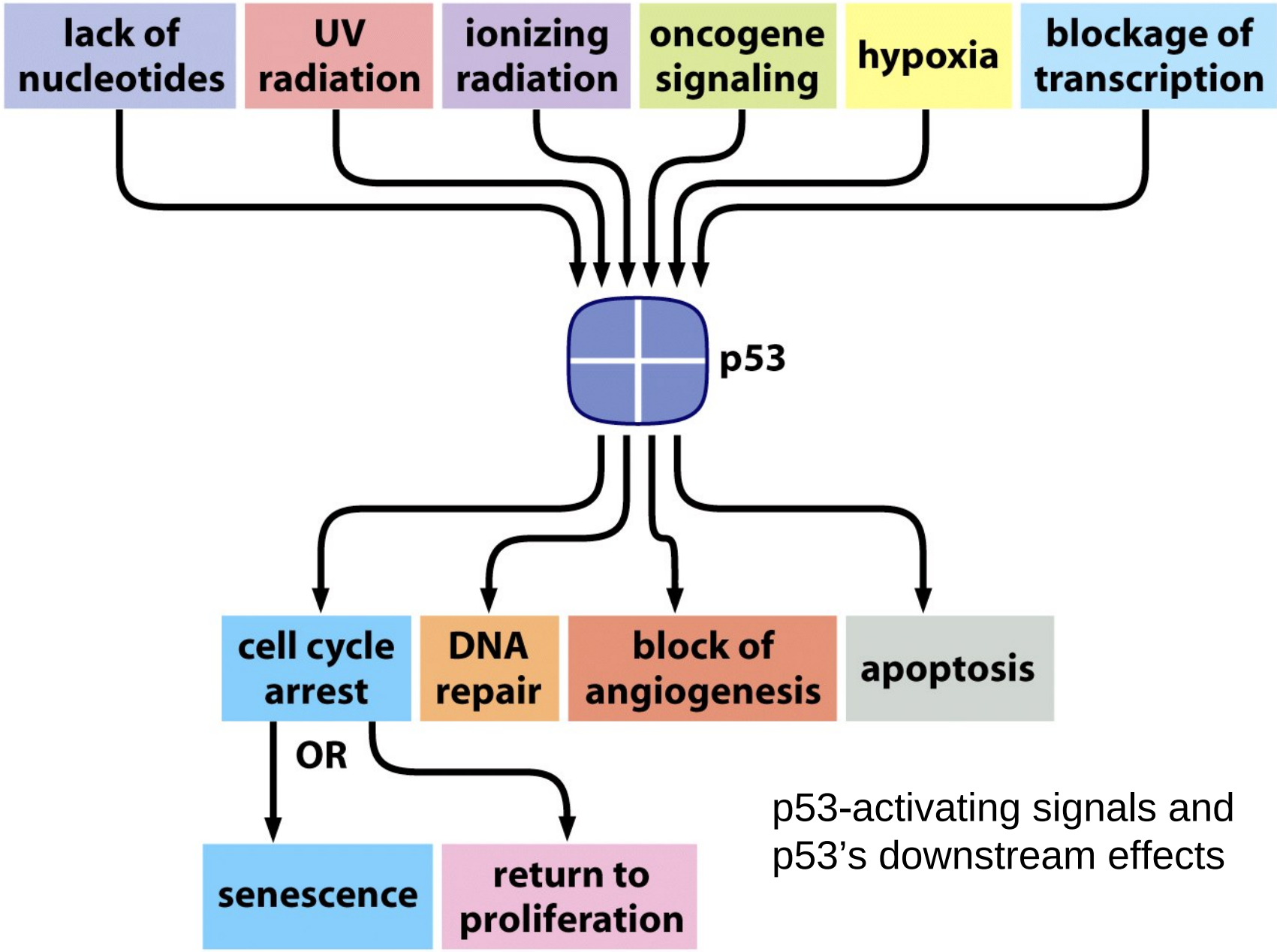

Figure 9-8 The Biology of Cancer (© Garland Science 2007)

p53-activating signals and p53's downstream effects

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 supressing 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.

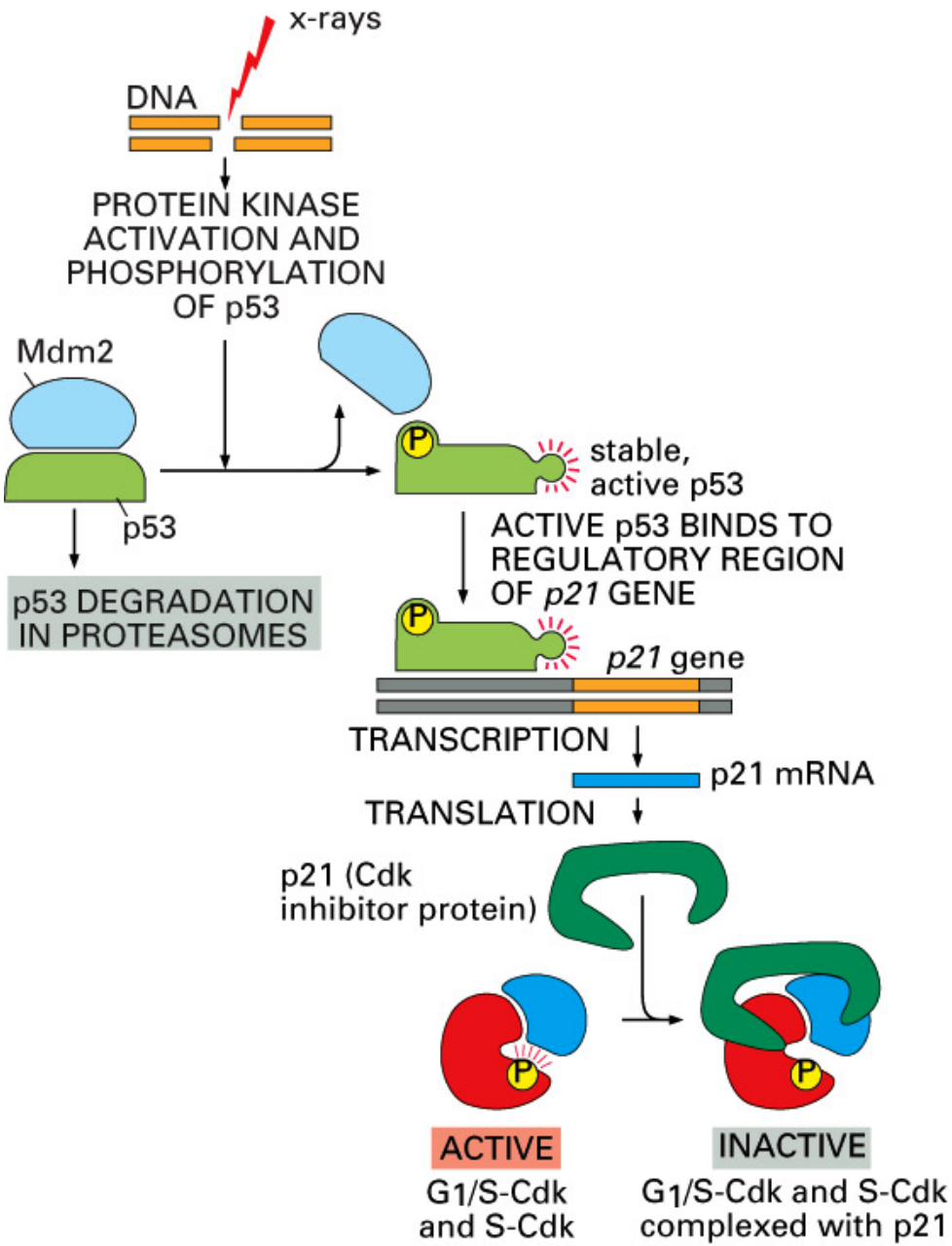

Figure 17–33. Molecular Biology of the Cell, 4th Edition.

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.

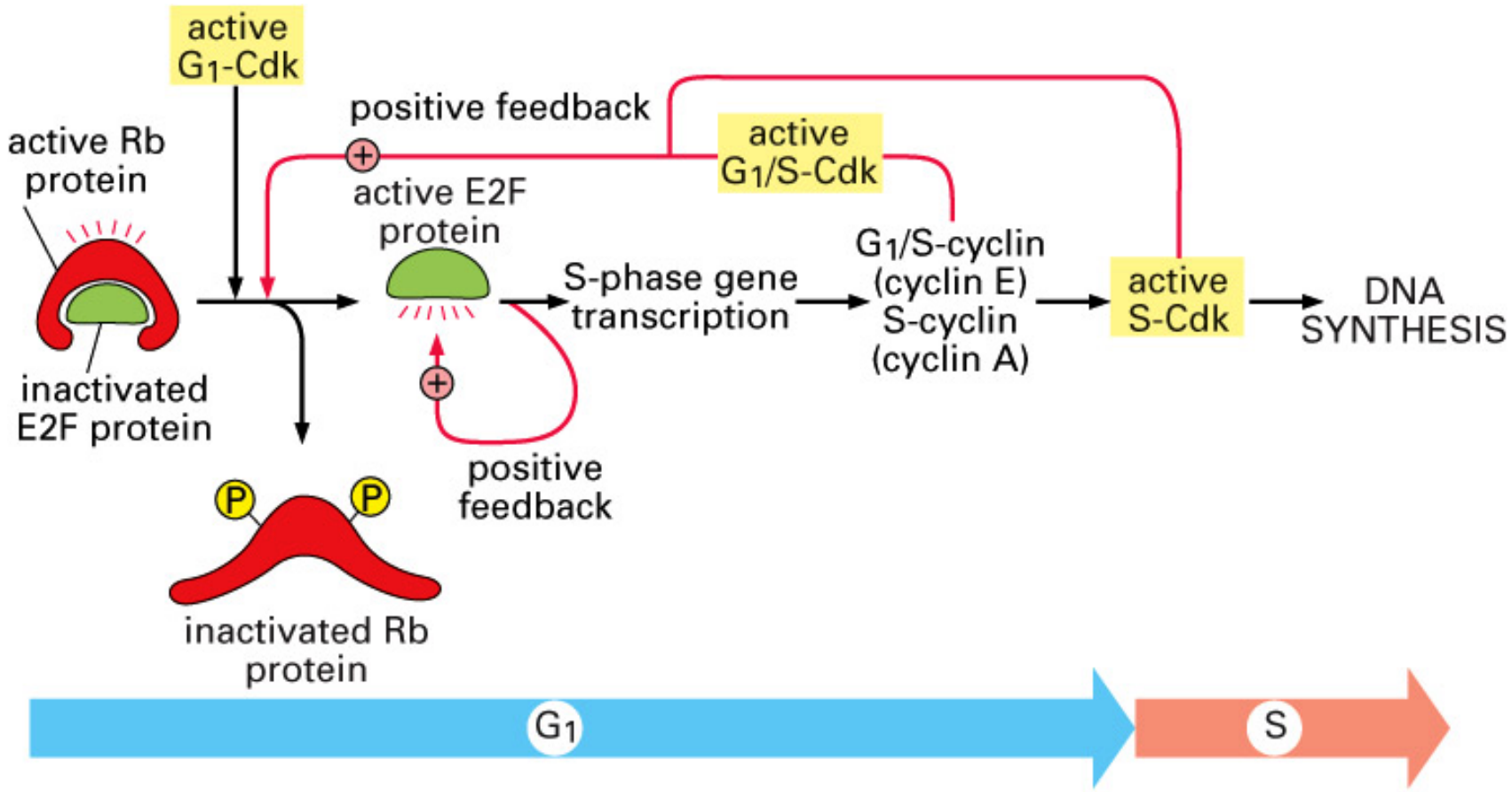

Figure 17–30. Molecular Biology of the Cell, 4th Edition.

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 phophoprotein whose expression and phosphorylation is cell cycle dependent.It is probably a DNA-binding transcription factor and also involved in DNA repair.

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.

Reference: Lomonosov et al., Genes Dev. 17: 3017-3022 (2003)

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.

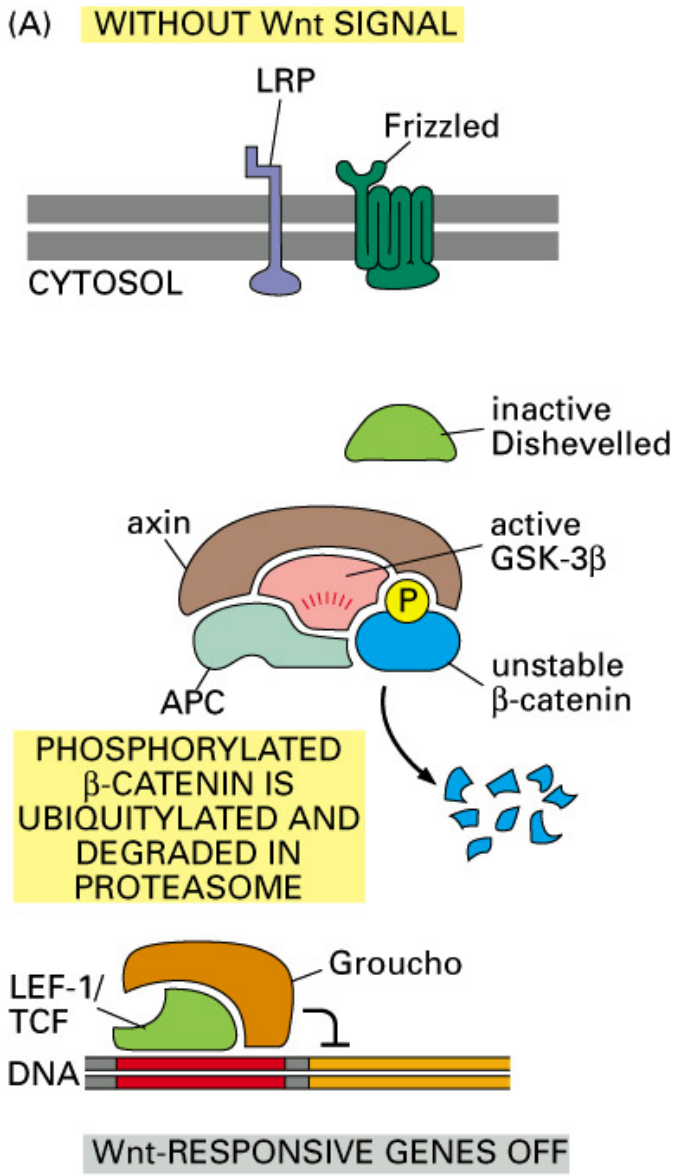

Figure 15–72 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

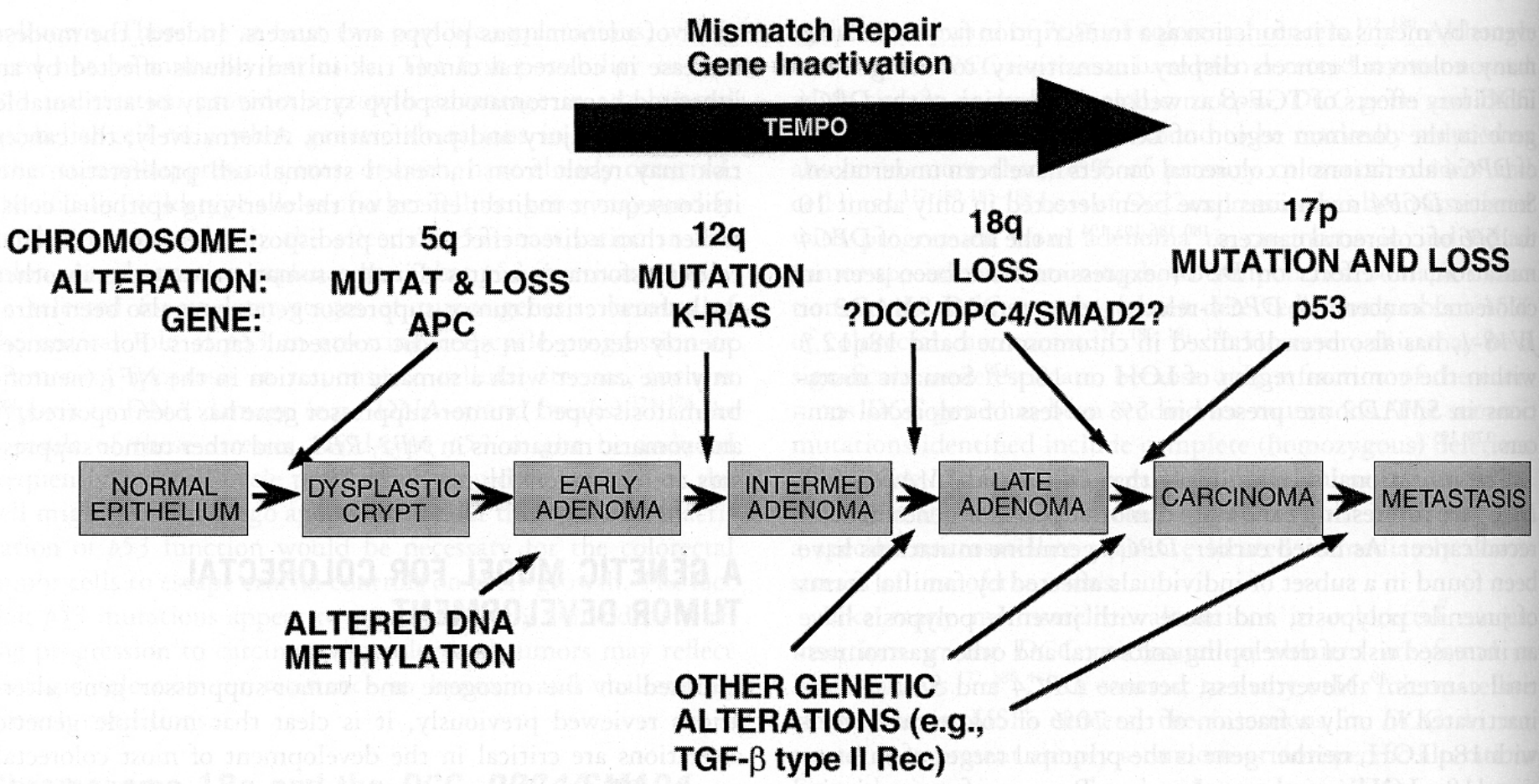

FIGURE 15–12. Genetic model of colorectal cancer. The majority of colorectal cancers are believed to arise from adenomatous polyps over a period of years or even decades. The inherited and somatic genetic alterations believed to underlie tumor initiation and progression are indicated and are discussed 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 pregression 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
RARβ2	nuclear receptor for differentiation	breast, lung
$p57^{Kip2}$	CDK inhibitor	gastric, pancreatic, hepatic; AML
TIMP3	inhibitor of metalloproteinases	diverse tumors
IGFBP	sequesters IGF-1 factor	diverse tumors
$CDKN2A/p16^{INK4A}$	inhibitor of CDK4/6	diverse tumors
$CDKN2B/p15^{INK4B}$	inhibitor of CDK4/6	diverse tumors
$p14^{ARF}$	inhibitor of HDM2/MDM2	colon, lymphoma
APC	inducer of β-catenin degradation	colon carcinomas
p73	aids p53 to trigger apoptosis	diverse tumors
GSTP1	mutagen inactivator	breast, liver, prostate
MGMT	DNA repair enzyme	colorectal
CDH1	cell–cell adhesion receptor	bladder, breast, colon, gastric
DAPK	kinase involved in cell death	bladder
MLH1	DNA mismatch repair enzyme	colon, endometrial, gastric
TGFBR2	TGF-β receptor	colon, gastric, small-cell lung
THBS1	angiogenesis inhibitor	colon, glioblastoma
RB	cell-cycle regulator	retinoblastoma
CASP8	apoptotic caspase	neuroblastoma, SCLC
APAF1	pro-apoptotic cascade	melanoma
CTMP	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

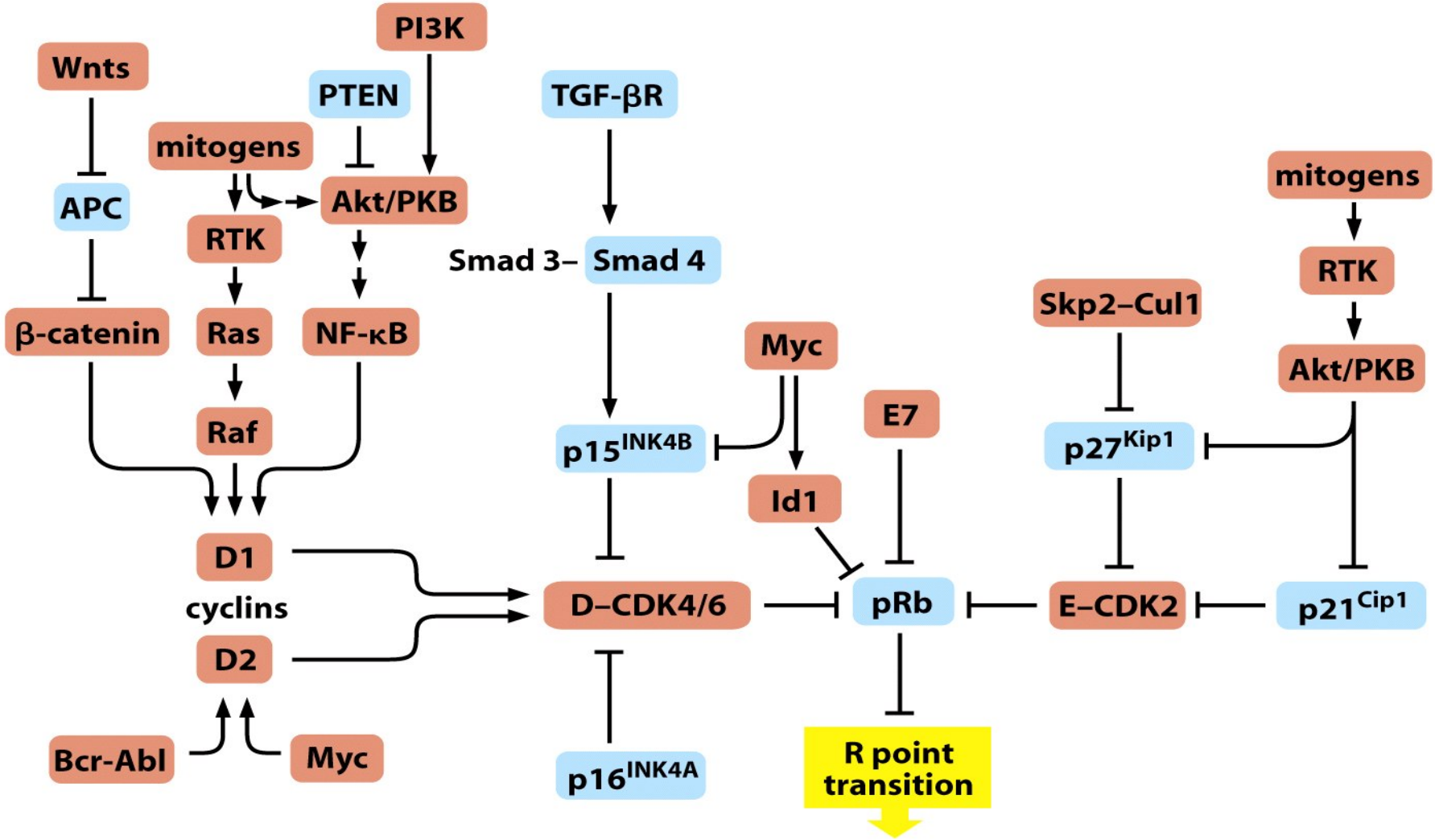

Figure 8-35 The Biology of Cancer (© Garland Science 2007)

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.

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).

Reference: J. Couzin. A new cancer player takes the stage. Science 310: 766-767, 2005.

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, Chapter 7, Garland Press, 2007.