

EDUCATION SESSION 7: MECHANISM OF CELL CONTROL



Signal Transduction and Leukemias

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I. Introduction

A number of oncogenes have been identified and isolated to date, and some of them encode cell growth factors, receptors for these growth factors, intracellular tyrosine kinases, serine/threonine kinases, and GTP-binding proteins (Fig. 1). Moreover, both the genes encoding transcription factors that regulate gene expression in the nucleus and the genes encoding cyclins that control the cell cycle are known to act as oncogenes when subjected to genetic alterations. Recently, apoptosis-regulating genes have also been suggested to be involved in human leukemias. The fact that some of these genes have been identified in the oncogenic forms of retroviruses for animals indicates that somatic mutations of these genes can play a role in tumorigenesis in humans. Mutations of some of these genes have actually been detected in human leukemias, indicating a close association between these mutations and the development of leukemias. Accordingly, it has come to be believed that mutations of any molecules involved in the signal transduction pathways from growth factors through receptors to inside the nucleus can lead to neoplastic transformation of hematopoietic cells. On the other hand, the existence of tu-

mor suppressor genes had been advocated on the basis of retinoblastoma and Wilm's tumor, in which specific chromosomal deletions are closely associated with their tumor development. Once the RB gene was isolated, it was found to be involved in a number of other types of cancers in addition to retinoblastoma. The p53 gene has also been identified as a tumor suppressor gene, and its inactivation has been detected in leukemias as well as solid tumors. The p16 gene, originally identified as a gene for an inhibitor of the cyclin D/CDK4 complex, has a negative regulatory role in G1 to S entry of the cell cycle control and was proved to be a tumor suppressor gene. Recently, it has been suggested that some of the genes encoding cell cycle regulators function as tumor suppressor genes. Here, signal transduction pathways will be reviewed and their lesions in human leukemia cells will be discussed.

2. Tyrosine Kinases

Chronic myelogenous leukemia (CML) is a hematopoietic disorder of pluripotent stem cells characterized by uncontrolled proliferation of the myeloid series in peripheral blood and bone marrow.⁽¹⁾ In the initial stage of the disease, chronic phase, the leukemic cells proliferate but have the ability to differentiate into mature granulocytes. However, the disease eventually accelerates after several years' duration and ultimately progresses to the terminal phase, blast crisis, which involves the accumulation of monoclonal immature hematopoietic cells arrested at an early stage of differentiation. The cytogenetic hallmark of the disease is the Philadelphia chromosome (Ph₁), a shortened chromosome 22. It is generated by a reciprocal translocation between chromosome 9 and 22, t(9;22)(q34;q11), where the c-ABL proto-oncogene on chromosome 9 is translocated into a 5.8 kilobase (kb) region on chromosome 22, denoted the major breakpoint cluster region (M-bcr).⁽²⁾ This translocation fuses the 5 exons of the BCR gene to most of the 3 exons of the c-ABL gene in a head-to-tail manner, thereby producing a novel 8.5kb chimeric BCR/ABL mRNA encoding a 210 kD protein (p210BCR/ABL).

The Ph₁ chromosome is also observed in 10% to 20% of acute lymphoblastic leukemia (ALL) patients. In approxi-

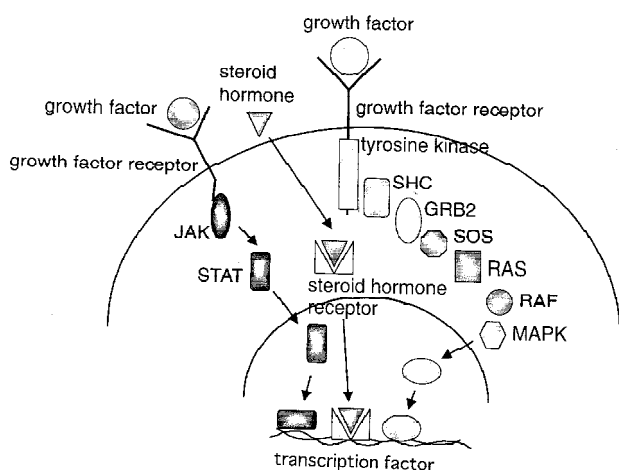


Figure 1. Signaling pathways

mately half of the cases, the translocation occurs at the same breakpoint as in CML patients and creates the p210BCR/ABL hybrid protein. However, in the remaining Ph₁-positive ALL patients, the breakpoint exists within the first intron of the BCR gene, designated the minor breakpoint cluster region (m-bcr). This event generates a 7.0 kb mRNA, which is transcribed to a 190 kD chimeric protein (p190BCR/ABL) with a smaller BCR moiety.^(3,4) Both of the chimeric proteins (p210BCR/ABL and p190BCR/ABL) possess enhanced kinase activity in comparison with the normal 145 kD c-ABL product and both have been considered to be implicated in the pathogenesis of Ph₁-positive human leukemias (Fig. 2).

BCR/ABL is a chimeric oncoprotein that exhibits deregulated tyrosine kinase activity and is implicated in the pathogenesis of Ph₁-positive human leukemias. Sequences within the first exon of BCR are required to activate the transforming potential of BCR/ABL. The SH2/SH3 domain-containing GRB-2 protein links tyrosine kinases to RAS signaling. It is shown that BCR/ABL exists in a complex with GRB-2 *in vivo*.⁽⁵⁾ Binding of GRB-2 to BCR/ABL is mediated by the direct interaction of the GRB-2 SH2 domain with a phosphorylated tyrosine, Y177, within the BCR first exon. The BCR/ABL-GRB-2 interaction is required for activation of the RAS signaling pathway (Fig. 3). Mutation of Y177 to phenylalanine (Y177F) abolishes GRB-2 binding and abrogates BCR/ABL-induced RAS activation. The BCR/ABL (Y177F) mutant is unable to transform primary bone marrow cultures and is impaired in its ability to transform Rat-1 fibroblasts. These findings implicate activation of RAS function as an important component in BCR/ABL-mediated transformation and demonstrate that GRB-2 not only functions in normal development and mitogenesis but also plays a role in oncogenesis.

The fused BCR sequences activate the tyrosine kinase, actin-binding, and transforming functions of ABL. Activation of the ABL transforming function has been shown to require two distinct domains of BCR: domain 1 (BCR amino acids 1 to 63) and domain 2 (BCR amino acids 176 to 242).

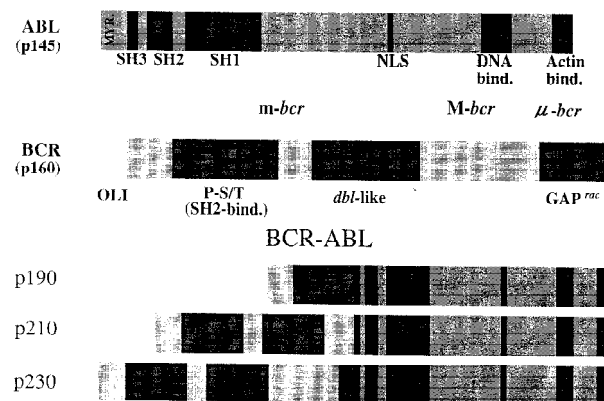


Figure 2. Schematic structure of BCR/ABL protein

The amino acid sequence of domain 1 indicates that it may be a coiled-coil oligomerization domain.⁽⁶⁾ It is revealed that domain 1 of BCR forms a homotetramer. Tetramerization of BCR/ABL through BCR domain 1 correlates with activation of the tyrosine kinase and F-actin-binding functions of ABL. Disruption of the coiled coil by insertional mutagenesis inactivates the oligomerization function as well as the ability of BCR/ABL to transform Rat-1 fibroblasts or to abrogate interleukin-3 dependence in lymphoid cells. These results strongly suggest that BCR/ABL oligomers are the active entities in transformation.

Biological function of the BCR/ABL oncogene is dependent on its activated tyrosine kinase. Mutations that inactivate the SRC homology 2 (SH2) domain, the GRB2-binding site in BCR, or the major autophosphorylation site of the kinase domain selectively disrupt downstream signaling but not tyrosine kinase activity. Despite a loss of fibroblast transformation activity, all three mutants retain the ability to render hematopoietic cell lines growth factor independent and transform primary bone marrow cells *in vitro*. *In vivo* tests of malignant potential reveal a most critical role for signals dependent on the BCR/ABL SH2 domain. The efficiency of both fibroblast and hematopoietic transformation by BCR/ABL is strongly affected by increased dosage of the SHC adapter protein, which can connect tyrosine kinase signals to RAS. The BCR/ABL oncogene activates multiple alternative pathways to RAS for hematopoietic transformation.⁽⁷⁾

3. GTP-Binding Proteins

The initial discovery that RAS genes endowed retroviruses with potent carcinogenic properties and the subsequent determination that mutated RAS genes were present in a wide variety of human cancers prompted a strong suspicion that the growth-promoting actions of mutated RAS proteins contribute to their aberrant regulation of growth stimulatory signaling pathways. In 1993, a remarkable convergence of experimental observations from genetic analyses of *Drosophila*, *S. cerevisiae* and *C. elegans* as well as biochemical and biological studies in mammalian cells came together to define a clear role for RAS in signal transduction. What emerged was an elegant linear signaling pathway where RAS

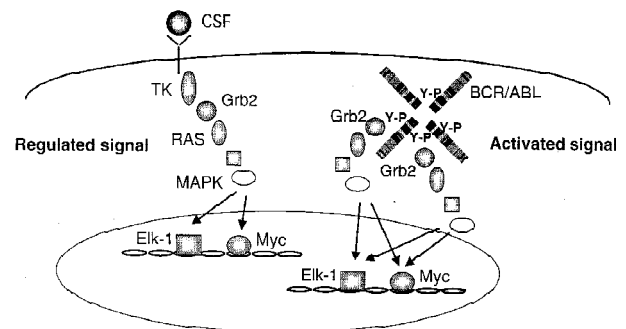


Figure 3. Proliferation signal by BCR/ABL

functions as a relay switch that is positioned downstream of cell surface receptor tyrosine kinases and upstream of a cytoplasmic cascade of kinases that included the mitogen-activated protein kinases (MAPKs). Activated MAPKs in turn regulated the activities of nuclear transcription factors. Thus, a signaling cascade where every component between the cell surface and the nucleus was defined and conserved in worms, flies, and man. This was a remarkable achievement in the efforts to appreciate how the aberrant function of RAS proteins may contribute to the malignant growth properties of the cancer cell. However, the identification of this pathway has proven to be just the beginning, rather than the culmination, of our understanding of RAS in signal transduction. Instead, we now appreciate that this simple linear pathway represents but a minor component of a very complex signaling circuitry. RAS signaling has emerged to involve a complex array of signaling pathways, where cross-talk, feedback loops, branch points and multi-component signaling complexes are recurring themes.⁽⁸⁾

GTP-binding proteins have both GTP-binding property and GTP-hydrolyzing activity, and are subjected to conformational change when they are converted from the GTP-binding form to the GDP-binding form, or from the GDP-binding form to the GTP-binding form. The GTP-binding form is the active form; it interacts with target proteins, and is converted to the GDP-binding form by hydrolysis of bound GTP. So far, a number of low-molecular weight GTP-binding proteins similar to p21 have been identified. Among them, RAS genes have been found to be involved in development of human hematopoietic tumors. RAS genes are revealed to be activated by a point mutation at amino acid codon 12, 13 or 61, and thought to contribute to tumorigenesis due to the resulting low GTPase activity (Fig. 4)⁽⁹⁾. RAS gene mutations have been noted in a large variety of malignant tumors including leukemias, but the mutation rate varies from tumor to tumor.

Mutations of the RAS gene are found frequently in adenocarcinomas of the pancreas (90%), the colon (50%), and the lung (30%); in thyroid tumors (50%); and in myeloid leukemia (30%), and T-cell tumors (30%), but rarely in the B-cell malignancies. For some tumor types a relationship

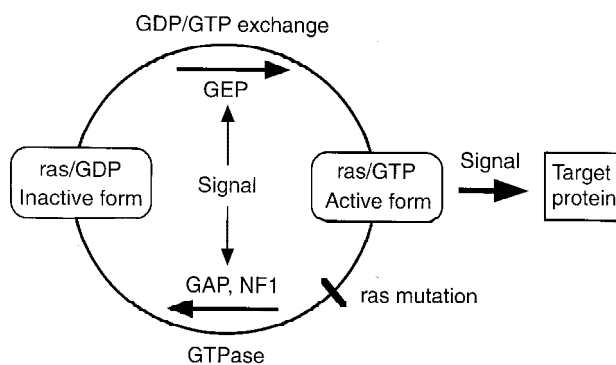


Figure 4. RAS protein cycle.

may exist between the presence of a RAS mutation and clinical or histopathological features of the tumor. There is some evidence that environmental agents may be involved in the induction of the mutations. Mutations of the RAS genes have also been detected in myelodysplastic syndrome (MDS), which is thought to be a preleukemic state. Such mutations are shown to have a role in progression from MDS to leukemia⁽¹⁰⁾. Of the three RAS genes, H-RAS, K-RAS, and N-RAS, mutations of N-RAS gene are most frequently detected in leukemic cells and bone marrow cells of MDS patients. RAS gene mutations are not disease-specific but are believed to play an important role in development of leukemia.

4. Transcription Factors

Understanding how pluripotent stem cells undergo progressive restriction of lineage potential and acquire the characteristics of mature, terminally differentiated cells is central to developmental biology. Accumulated evidence suggests that transcription factors have important roles in asymmetric cell division and hematopoietic lineage commitment resulting in specifying hematopoietic cell types (Fig. 5). Hematopoiesis is the process by which hematopoietic cells acquire defining phenotypes as a result of coordinated, cell-specific gene expression. The pattern of gene expression within a cell is established by cell-specific transcription factors that mediate the net effect of the variety of proliferation and differentiation signals. Therefore, understanding the function of transcription factors is essential to study hematopoiesis. Such roles of transcription factors in hematopoiesis have been analyzed by the method of targeted gene disruption in mice. Recent studies have shown that hematopoietic transcription factors can engage in multiple protein-protein interactions and that specific complexes define differentiation lineages and stages. Leukemia is an acquired genetic disease caused by accumulation of chromosomal abnormalities and genetic mutations which modify either the biochemical property or the level of expression of proteins. Since the genes rearranged in chromosomal translo-

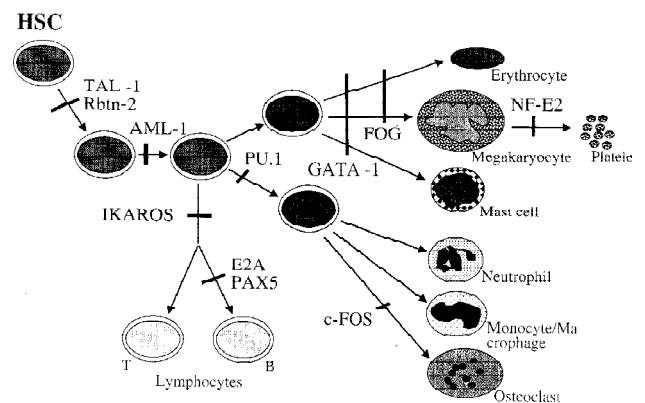


Figure 5. Hematopoiesis cell differentiation and transcription factors.

cations frequently encode transcription factors, it has been considered that transcription factors also have important roles in leukemogenesis.

(1) Retinoid signaling pathway

Retinoids play an important role in development, differentiation, and homeostasis. The discovery of retinoid receptors belonging to the superfamily of nuclear ligand-activated transcriptional regulators has revolutionized our molecular understanding as to how these structurally simple molecules exert their pleiotropic effects. Diversity in the control of gene expression by retinoid signals is generated through complexity at different levels of the signaling pathway. A major source of diversity originates from the existence of two families of retinoic acid (RA) receptors (R), the RAR isotypes (alpha, beta, and gamma) and the three retinoid X receptor (RXR) isotypes (alpha, beta, and gamma), and their numerous isoforms, which bind as RXR/RAR heterodimers to the polymorphic cis-acting response elements of RA target genes. The possibility of cross-modulation (cross-talk) with cell-surface receptors signaling pathways, as well as the finding that RARs and RXRs interact with multiple putative coactivators and/or corepressors, generates additional levels of complexity for the array of combinatorial effects that underlie the pleiotropic effects of retinoids.⁽¹¹⁾

Among transcription factors, steroid receptors are unique in that they become activated when bound to steroids and bind to the specific nucleotide sequence that serves as a transcription enhancer, thereby increasing the transcription level of the target genes. An example of abnormal regulation of this transcription mechanism is the t(15;17) translocation found in APL. The t(15;17) translocation is observed in 70% or more of the APL patients. The genes at the breakpoint of this translocation have been cloned, and it has been proved that the recombination between the PML gene on chromosome 15 and the RAR α chain (RAR α) gene on chromosome 17 results in the generation of the PMURAR α chimeric gene^(12,13). However, a small subset of APL are patients have a different fusion gene, promyelocytic leukemia zinc finger (PLZF)-RAR α , resulting from the

variant translocation t(11;17)(q23;21).⁽¹⁴⁾ The other chimeric genes, nucleophosmin (NPM)/RAR α in t(5;17) and nuclear matrix-associated antigen (NuMA) in t(11;17)(q13;q21), have also been described.^(15,16) The individuals with PML/RAR α can be successfully treated with all-trans retinoic acid (ATRA), resulting in the differentiation of immature promyelocytes into mature granulocyte forms and elimination of the malignant clone, and leading to complete remission. Similarly, promyelocytes from a patient with t(5;17) and NPM-RAR expression can undergo terminal granulocytic differentiation in vitro. A patient with a NuMA-RAR fusion and t(11;17)(q13;q21) also clinically responded to ATRA therapy.

The RAR α gene product is a transcription factor belonging to the steroid hormone/thyroid hormone receptor family. As a result of binding with retinoic acid, it becomes bound to specific consensus sequence in the upstream of various genes. All-trans retinoic acid induces complete remission of APL by its differentiation-inducing action through RAR α , but the presence of the RAR α chimeric protein is essential for this effect. Since the RAR α chimeric protein has a dominant negative effect on the function of RAR α , it appears to suppress the transcription activity of normal RAR α in the absence of retinoic acid, thereby resulting in block of myeloid differentiation otherwise induced by normal RAR α . The clinical effects of all-trans retinoic acid can be explained by elimination of the dominant negative effect of the RAR α chimeric protein.

In striking contrast, t(11;17)(q23;q21)-associated APL is not responsive to ATRA therapy⁽¹⁷⁾, and no patients with this disease have achieved lasting clinical remission with conventional chemotherapy (Fig. 6). In all four forms of APL, an aberrant RAR is generated, perhaps leading to the promyelocytic phenotype due to a block in RA-mediated signaling. While PML-RAR can activate these genes after treatment with pharmacological doses of ATRA, PLZF-RAR fails to activate such genes due to the ability of the PLZF moiety of the fusion protein to interact with SMRT and N-CoR corepressor proteins even in the presence of ATRA^(18,19). Thus, part of the resistant phenotype of t(11;17)-associated

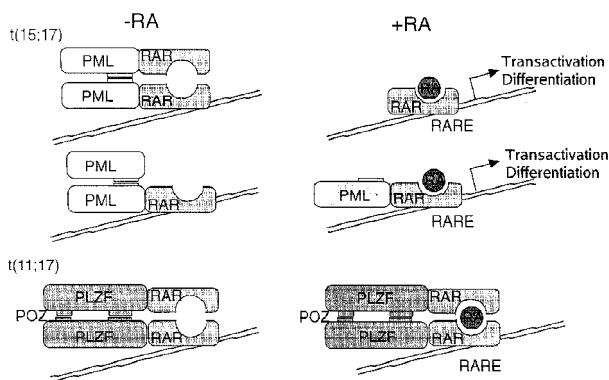


Figure 6. Differentiation therapy for APL.

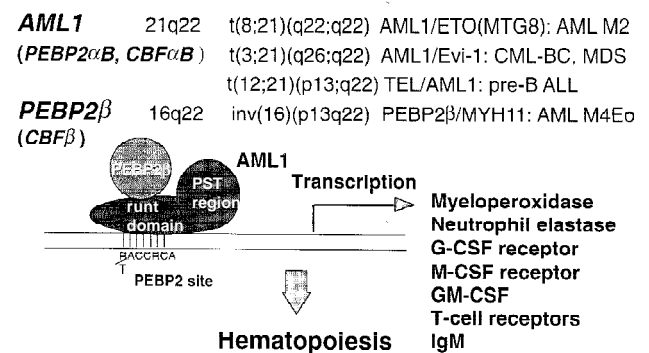


Figure 7. AML1 transcription factor and leukemias.

APL may be due to a reduced capacity of the PLZF-RAR chimeric protein to transduce ATRA-mediated differentiation signals. However, a transgenic model for t(11;17) APL yielded a myeloid leukemia that did respond to high-dose ATRA with an increase in differentiated granulocyte forms, suggesting that in the organism, PLZF-RAR, though leukemogenic, does not completely block differentiation. The therapeutic effect of ATRA in induction of remission for APL has largely been proved, and this has, over the past 10 years, greatly stimulated research on oncogenesis and RA-regulated differentiation pathways.

(2) AML1 transcription factor

The AML1 gene was first identified as the gene on chromosome 21 which is disrupted in the (8;21)(q22;q22) translocation associated with acute myelogenous leukemia (AML) (Fig. 7). In t(8;21)(q22;q22), the gene rearrangement results in the production of an AML1/ETO(MTG8) fusion protein.^(20,21) The AML1 gene also forms chimeric genes, AML1/Evi-1 in t(3;21)(q26;q22) translocation associated with blastic crisis of chronic myelogenous leukemia and myelodysplastic syndrome, and TEL/AML1 in acute lymphoblastic leukemia carrying t(12;21)(p12;q22).(22,23) PEBP2 α B/CBF α 2, which is a mouse homolog of AML1, was first identified as the gene encoding a member of the polyomavirus enhancer binding protein (PEBP) 2 α family or a core binding factor (CBF) of Molony leukemia virus enhancer. PEBP2 α /CBF α and PEBP2 β /CBF β are components of the PEBP2/CBF heterodimer, which binds to the cores of polyomavirus and Molony leukemia virus enhancers. The mammalian PEBP2 α /CBF α subunits are encoded by three distinct genes; AML1 (PEBP2 α B/CBF α 2), AML2 (PEBP2 α C/CBF α 3), and AML3 (PEBP2 α /CBF α 1). A human homolog of PEBP2 β /CBF β generates PEBP2 β (CBF β)/SMMHC fusion in inv(16)(p13q22) associated with acute myelogenous leukemia (FAB classification M4Eo)⁽²⁴⁾, suggesting critical roles of both PEBP2/CBF heterodimer components in leukemogenesis.

AML1 has been shown to regulate the expression of several hematopoietic lineage-specific genes, such as those for myeloperoxidase, leukocyte elastase, macrophage colony-stimulating factor (colony-stimulating factor 1) receptor, granulocyte-macrophage colony-stimulating factor, and T-cell receptors. We have shown that AML1 regulates myeloid cell differentiation and transcriptional activation antagonistically by two alternative spliced forms, suggesting that a transactivation property of AML1 is necessary for myeloid cell differentiation.⁽²⁵⁾ It was recently shown that mice lacking AML1 or PEBP2 β (CBF β) die during midembryonic development due to extensive hemorrhaging in the central nervous system and show the complete absence of definitive hematopoiesis.^(26,27) These findings suggest that AML1 contributes, by regulating the expression of target genes, to hematopoietic cell differentiation and proliferation.

Within the AML1 protein, two functional domains have been identified. The runt domain, a 128-amino acids region

of homology with the *Drosophila* runt protein, is known to be essential for DNA binding and heterodimerization with PEBP2 β /CBF β . AML1 specifically recognizes a consensus sequence, designated as a PEBP2 site, while PEBP2 β /CBF β binds to AML1 and increases its affinity for DNA without interacting with DNA by itself. The proline-, serine-, threonine-rich (PST) region is essential for transcriptional activation, and this region is missing in the chimeric proteins AML1/ETO(MTG8) and AML1/Evi-1. It is demonstrated that AML1 is phosphorylated in vivo on two serine residues within the PST region with dependence on extracellular signal-regulated kinase (ERK) activation.⁽²⁸⁾

The biological activity of AML1/Evi-1 fusion protein in leukemogenesis was analyzed employing 32Dcl3 murine IL-3-dependent myeloid cell line, which clearly differentiates to mature granulocytes when treated with granulocyte colony-stimulating factor (G-CSF). The results demonstrate that the AML1/Evi-1 fusion protein blocks terminal differentiation to mature granulocytes and stimulates cellular proliferation in 32Dcl3 cells.⁽²⁹⁾ In accordance with this biological property, the transactivation abilities of AML1 through the PEBP2 site are reduced in the presence of AML1/Evi-1 protein, indicating that AML1/Evi-1 dominantly and specifically suppresses the transactivation by AML1. It was revealed that AML1/Evi-1 as well as AML1 specifically binds to a PEBP2 site. The affinity of AML1/Evi-1 for the PEBP2 site was several-fold higher than that of AML1, supporting the hypothesis that AML1/Evi-1 dominantly suppresses AML1 transactivation by competing with AML1 for binding to the PEBP2 site.⁽²⁹⁾ In addition to this evidence, the dominant negative effects of these AML1 chimeras are also supported by the fact that only one allele of AML1 is altered in leukemic cells expressing t(3;21) or t(8;21). Interestingly, AML1/ETO(MTG8) and PEBP2 β (CBF β)/SMMHC knock-in mice display a phenotype similar to that of AML-1- and PEBP2 β (CBF β)-deficient mice, as they die during embryogenesis from central nervous system hemorrhages and exhibit severe blocks in fetal liver hematopoiesis^(30,31). These data suggest that AML1 is an important regulator of hematopoiesis.

Cell Cycle Regulators

Genetic lesions found in tumors are often targeted to the negative growth regulatory tumor suppressor genes. Much of our understanding of tumor suppressor gene function is derived from experimental manipulations in cultured cells. Recently, however, the generation of mice with germ line tumor suppressor gene mutations through gene targeting in embryonic stem cells has provided another dimension by allowing experimental studies of tumor suppressor function in an organismal context. Novel insights into the role of tumor suppressors in development, differentiation, cell cycle control, and tumor suppression have been obtained from the studies on these 'knockout' mice. In addition, such mice may serve as disease models for humans with inherited cancer predisposition syndromes. Perhaps the greatest advantage of many of the mouse tumor suppressor models

is that they facilitate study of the roles of tumor suppressor gene loss in tumor initiation and progression in vivo. Moreover, derivation of primary cells from tumor suppressor-deficient mice has provided an important resource for in vitro studies on the role of targeted genes in cell cycle regulation, DNA damage response, regulation of apoptotic pathways, and preservation of genomic stability.

Recently, advances in understanding of cell cycle regulation have provided a new insight into its roles in human oncogenesis. For years, it has been established that dysregulation of the cell cycle machinery could contribute to development of human cancers.⁽³²⁻³⁴⁾ Overexpression of cyclin D, a mammalian G1 cyclin, is observed in a variety of tumors, including parathyroid tumors, esophageal cancers, centrocytic lymphomas, and breast cancers. Because cyclin D binds to its partner, CDK4 (or CDK6), to form a cyclin D/CDK4 (or CDK6) complex, and acts as a positive regulator for the cell cycle through phosphorylation of Rb protein, its overexpression is considered to facilitate G1 to S entry and to contribute to tumorigenesis. On the other hand, discovery of diverse negative regulatory elements in the cell cycle machinery has led to another idea that these negative regulators of the cell cycle may potentially act as tumor suppressors, inactivation of which may be associated with cancer development. Thus far, various molecules have been implicated in negative regulation of the cell cycle. Of particular interest are cyclin-dependent kinase inhibitors (CDKIs), a novel family of cell cycle regulatory mol-

ecules, including p21Cip1, p27Kip1, p57Kip2, and four members of the INK family: CDKIs, p16INK4A, p15INK4B, p18,19 and p19. They were presumed to exert negative regulatory effects on the cell cycle through binding to G1 cyclin-bound CDKs (**Fig. 8**). A feedback regulatory loop involving pRb, p16INK4A, and CDKs regulates G1/S phases transition. p16INK4A and p15INK4B are deleted in high frequency in human cell lines and in some fresh solid tumors. Point mutations of p16INK4A have also been sequenced, especially in familial melanomas and digestive cancers, but the preferential mechanism of p16INK4A/p15INK4B inactivation seems to be due to biallelic deletion. In hematological malignancies, homozygous deletions of p16INK4A and p15INK4B occur frequently in acute lymphoblastic leukemia (ALL) (14-40%), lymphoid type blast crisis of CML, and adult T cell leukemia (ATL). p16INK4A deletions are more frequent than p15INK4B deletions, and hemizygous deletions of either p16INK4A and p15INK4B are rare.⁽³⁵⁻³⁹⁾ These results suggest that the p16 gene has an essential role in the development of lymphocytic tumors.

p53, a well-characterized tumor suppressor protein, is also known to participate in negative regulation of the cell cycle. It induces a member of CDKIs, p21Cip1, in response to DNA damage and the p21Cip1 blocks entry of a cell from G1 to S. It is now established that the p53 protein is central to the cellular response to a wide variety of stressful stimuli. These stimuli, which include DNA damage, hypoxia, heat shock, metabolic changes and certain cytokines, activate

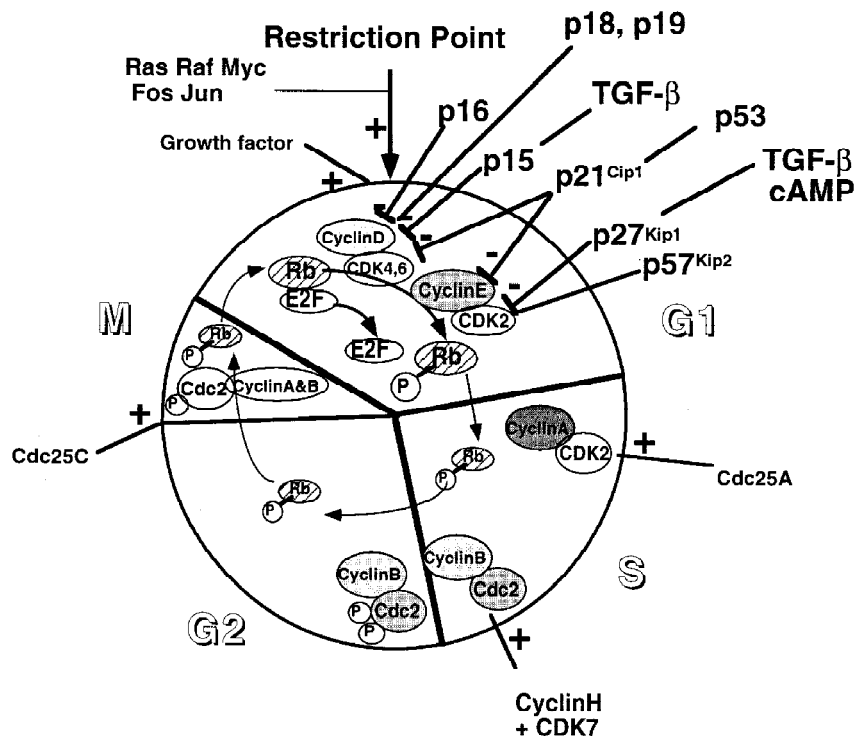


Figure 8. Cell cycle regulators.

the p53 protein, which in turn drives a series of events that culminate either in cell cycle arrest or apoptosis (programmed cell death). Disruption of the p53 pathway significantly affects the ability to repair or discard a damaged cell, which can then go on to replicate. If this cell has sustained damage to one or more proto-oncogenes or to other tumour suppressor genes, a cancer may result. So one of the physiological roles of p53 is to prevent the formation of tumours and damage to the p53 gene itself. Inactivation of the p53 gene through deletion, mutation, or insertion on both alleles is believed to result in malignant transformation of the cells. Alterations of the p53 gene have been widely observed in a variety of human cancers, and the p53 gene is most frequently involved in cancers. Mutations of the p53 gene have been reported in families with Li-Fraumeni syndrome.⁽⁴⁰⁾ Among hematopoietic tumors, the p53 gene mutations have frequently been detected in cells of blast crisis in CML, suggesting its association with the blast crisis of CML.⁽⁴¹⁾ p53 gene mutations become more frequent along with the increasing cell proliferation potential, as seen in the order of MDS patients, leukemia patients, and leukemia cell lines.⁽⁴²⁻⁴⁴⁾ p53 gene mutations have also been observed in advanced stages of MDS. All these findings strongly suggest that p53 inactivation confers oncogenic potential on the hematopoietic cells.

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