

REVIEW

Role of tumor suppressor genes in the development of adult T cell leukemia/lymphoma (ATLL)

Y Hatta¹ and HP Koeffler²

¹First Department of Internal Medicine, Nihon University School of Medicine, Tokyo, Japan; and ²Division of Hematology/Oncology, Cedars-Sinai Medical Center, UCLA School of Medicine, Los Angeles, CA, USA

Adult T cell leukemia/lymphoma (ATLL) is one of the peripheral T cell malignant neoplasms strongly associated with human T cell leukemia virus type-I (HTLV-I). Although the viral transactivating protein Tax has been proposed to play a critical role in leukemogenesis as shown by its transforming activity in various experimental systems, additional cellular events are required for the development of ATLL. One of the genetic events in ATLL is inactivation of tumor suppressor genes. Among many candidates for tumor suppressor genes, the main genetic events have been reported to center around the cyclin-dependent kinase inhibitors ((CDKIs) p15^{INK4A}, p16^{INK4B}, p18^{INK4C}, p19^{INK4D}, p21^{WAF1}, p27^{KIP1}, and p57^{KIP2}), p53 and Rb genes; all of them play a major regulatory role during G1 to S transition in the cell cycle. Acute/lymphomatous ATLL has frequent alterations of p15 (20%) and p16 (28–67%), while chronic/smoldering ATLL has fewer abnormalities of p15 (0–13%) and p16 (5–26%). Most of these changes are deletion of the genes; fewer samples have mutations. ATLL patients with deleted p15 and/or p16 genes have significantly shorter survival than those individuals with both genes preserved. Although genetic alterations of p18, p19, p21, p27 have rarely been reported, inactivation of these genes may contribute to the development of ATLL because low expression levels of these genes seem to mark ATLL. The p53 gene is mutated in 10–50% of acute/lymphomatous ATLL. Functional impairment of the p53 protein, even if the gene has wild-type sequences, has been suggested in HTLV-I infected cells. Each of these genetic events are mainly found in acute/lymphomatous ATLL, suggesting that alterations of these genes may be associated with transformation to an aggressive phenotype. The Rb tumor suppressor gene is infrequently structurally altered, but one half of ATLL cases have lost expression of this key protein. Notably, alterations of one of the CDKIs, p53 and Rb genes appear to obviate the need for inactivation of other genes in the same pathway. A novel tumor suppressor gene on chromosome 6q may also have a critical role in the pathogenesis of ATLL. Taken together, tumor suppressor genes are frequently altered in acute/lymphomatous ATLL and their alteration is probably the driving force fueling the transition from chronic/smoldering to acute/lymphomatous ATLL.

Leukemia (2002) 16, 1069–1085. DOI: 10.1038/sj/leu/2402458

Keywords: ATLL; tumor suppressor gene; cyclin-dependent kinase inhibitor; Tax

Introduction

Adult T cell leukemia/lymphoma (ATLL) is an aggressive, fatal malignancy of mature CD4⁺ T lymphocytes.^{1–4} The retrovirus, human T cell lymphotropic virus type I (HTLV-I), has been recognized as the etiologic agent of ATLL.^{5–8} HTLV-I is endemic in Southern Japan and the Caribbean basin. Thus,

ATLL is prevalent in these areas,⁹ and occurs sporadically in Africa, Latin America, the Middle East and the United States.^{10–14} ATLL has been classified into four main subtypes.¹⁵ In the relatively indolent smoldering and chronic forms, the median survival is 2 years or more. In the acute and lymphomatous forms, the median survival time is about 13 months.¹⁶ Because hematopoietic stem cell transplantation combined with chemotherapy has been applied for acute/lymphomatous ATLL, the survival rate will be progressed. Transformation from chronic to acute type occurs frequently. Acute and lymphomatous ATLL is characterized by high white blood cell counts with convoluted nuclei, hepatosplenomegaly, lymphadenopathy, skin lesions, frequent hypercalcemia, and a rapidly progressive terminal course.^{1,17–19} HTLV-I is also associated with chronic inflammatory disorders such as tropical spastic paraparesis (TSP/HAM)/HTLV-I-associated myelopathy, HTLV-I-associated arthropathy, alveolitis, dermatitis and uveitis.^{20–25}

HTLV-I contains no typical oncogenes.^{26–29} The viral factor of HTLV-I responsible for induction of leukemia is proposed to be Tax, a regulatory protein encoded by the pX sequence of the virus,³⁰ which immortalizes human T cells and induces tumors in transgenic mice.^{31–33} Tax transcriptionally activates the expression of certain cellular genes, including interleukin-2 (IL-2), IL-2 receptor (IL-2R), IL-8,³⁴ IL-10,³⁵ granulocyte-macrophage colony-stimulating factor (GM-CSF), and several proto-oncogenes. On the contrary, Tax downregulates the DNA repair enzyme β -polymerase.³⁶ However, the role of Tax in leukemogenesis has not been precisely defined because of the following observations. The HTLV-I virus integrates randomly into the genomic DNA; the sites of insertion show that the transformation process can not be explained solely by the activation of proto-oncogenes by insertional mutagenesis or disruption of tumor suppressor genes.^{37,38} Transcripts of tax have been detected in the peripheral blood mononuclear cells (PBMNCs) in about 20% of asymptomatic carriers by nested RT-PCR,^{1,39} whereas ATLL cells usually do not express viral proteins including Tax.⁴⁰ These findings indicate that HTLV-I may be merely one of the factors in leukemogenesis of ATLL. Evidence that additional cellular events are required for the development of the malignant phenotype includes: (1) a long period of clinical latency (mean 55 years) precedes the development of ATLL;⁴¹ (2) only a small percentage of HTLV-I infected individuals develop this malignancy (one patient/1000–2000 carriers/year);^{41,42} (3) leukemic cells of ATLL are monoclonal;^{5,43} and (4) a statistical analysis revealed that five independent genetic events were probably required to develop ATLL after viral infection of the target T cells.⁴⁴

Few details are known concerning the genetic events leading to acute/lymphomatous from chronic/smoldering ATLL. As we will show, the weight of evidence suggests that alterations of tumor suppressor genes lead to the progression to ATLL. An

Correspondence: Y Hatta, First Department of Internal Medicine, Nihon University School of Medicine, 30–1 Oyaguchi, Itabashi-ku, Tokyo, 173–8610, Japan; Fax: 81–3–3972–2893

Received 1 December 2001; accepted 31 December 2001

overview of the data presented in this review is given in Tables 1 and 2.

Short overview of the cell cycle

Recently, the control of the cell cycle in mammalian cells has been carefully analyzed, and its relationship to development and progression of cancer is beginning to be elucidated. Derangement of cell cycle control can lead to uncontrolled cell growth and malignant transformation.⁴⁵

A family of serine/threonine kinases known as cyclin-dependent kinases (CDKs) regulates cell cycle progression in eukaryotes.^{46–50} Mammalian cells contain at least seven CDKs (CDK1–7)^{49,51,52} whose protein levels are constant throughout the cell cycle. The CDKs are either activated or inactivated at specific time points during the cell cycle.⁴⁸ Activation of CDKs requires their association with the regulatory subunits called cyclins. Twelve cyclins (A, A1, B1, B2, C, D1, D2, D3, E, F, G and H)^{52,53} have been described, which are expressed in a cell cycle-dependent manner. Among them, D-type cyclins (D1, D2 and D3) and cyclin E are involved in regulating progression through G1 and entry into S phase, and therefore are termed G1 cyclins.^{54–57} The half-life of these cyclins is short (approximately 30 min). Ectopic expression of cyclin D1 accelerates the entry into S phase.^{58,59} D-type cyclins are associated mainly with either CDK4 or CDK6.^{54,57,60} Cyclin E binds to CDK2.⁶¹ Once cells enter S phase, cyclin E is degraded, and CDK2 forms complexes with cyclin A. This complex functions at least during the S phase.⁶²

The major substrate for the cyclin D–CDK complex is the retinoblastoma protein (pRb), a key tumor suppressor protein.^{63,64} During G0 and early G1, the Rb protein binds and inactivates a key family of transcription factors known as E2F.⁶⁵ At mid to late G1, Rb becomes extensively phosphorylated by CDK4/CDK6 which causes release of the E2F family of proteins from Rb. The E2F proteins play an important role in transactivating genes associated with DNA synthesis and cell division such as dihydrofolate reductase, thymidine kinase and *c-myc*. Thus, phosphorylation of pRb reverses its growth suppressive function and enables the activation of genes coding transcription factors that are key to the S phase of the cell cycle.^{65,66} Several oncoproteins of DNA tumor viruses, including E1A of adenovirus, E7 of papilloma virus, and large T of SV-40 virus, bind directly to and inactivate the tumor suppressor pRb to drive quiescent cells into a replicative state which may lead to cellular immortalization.^{65,67,68}

The overexpression of cyclin D is found in some types of lymphoma/leukemia cases and is the *sine quo non* of mantle cell lymphoma. Akagi *et al*⁶⁹ reported that cyclin D2 was expressed at a high level in HTLV-I infected T cell lines and was not expressed in T cell lines not infected with the virus. By contrast, HTLV-I infected T cell lines expressed CDK4 and CDK6 at lower levels.⁶⁹ However, whether HTLV-I uniformly enhances cyclin D and suppresses CDKs is unclear because in another study, expression levels of cyclin D1, D2 and D3 and CDK4 were not altered in T cells by Tax or HTLV-I infection.⁷⁰

Cyclin-dependent kinases (CDKs) and tumorigenesis

Cell cycle progression in eukaryotic cells is regulated by sequential activation and inactivation of CDKs.⁵⁵ The activi-

ties of CDKs are positively regulated by cyclins and negatively regulated by cyclin-dependent kinase inhibitors (CDKI) at different stages of the cell cycle.⁵⁰ Because all of the CDKs block entry into S phase and thus inhibit initiation of DNA synthesis and proliferation, each are candidate tumor suppressor genes.

The CDKs can be classified into two groups based on their structure. p15^{INK4B},⁷¹ p16^{INK4A},⁷² p18^{INK4C},^{73,74} and the more recently described p19^{INK4D},^{74,75} which have ankyrin repeat motifs, belong to one group of CDKs known as the INK4 family. p16 is the first described gene in the INK4 family and is also known as MTS1 and CDKN2. The official HUGO nomenclature is CDKN2; however, the acronym p16 is much more widely used. The CDKs of the INK4 family bind to CDK4 and CDK6 and inhibit their kinase activities, thereby resulting in arrest of the cell cycle.^{72–75} Mutations of p16 outside of the ankyrin repeats usually do not affect the function of the p16 protein *in vitro*.⁷⁶ Therefore, the ankyrin repeats are probably involved in the binding of INK4 family of proteins to CDK4 and CDK6. Expression of p16 was able to suppress the transformed phenotype of cells induced by H-Ras and C-Myc,⁷⁷ and to produce G1 arrest in cell lines which retain functional pRb.^{73,78–81}

The p16 shares a great deal of nucleotide sequence homology with p15, which was identified as an inhibitor induced by transforming growth factor- β (TGF- β).⁷¹ The p15 gene is localized 25 kb upstream of the p16 locus on chromosome 9p21.^{82,83} This entire region is associated with chromosomal rearrangements and loss in many tumors.^{84–89} The p15 and p16 CDKI genes are very frequently altered, such as deletion and mutation, in a variety of tumors including hematologic malignancies.^{90–93} These aberrations are considered to contribute greatly to the abnormal growth properties of these tumor cells.^{45,85,94–97} Thus, both p15 and p16 are suggested as acting as tumor suppressors whose inactivation contributes to the development of human tumors.

The biochemical and biological properties of p18 and p19 suggest that they could have tumor suppressor functions similar to the p15 and p16 genes.^{73–75} The genes for p18 and p19 are located at 1p32 and 19p13, respectively.^{73,75} Although chromosome 1p32 is frequently rearranged in a variety of solid tumors, almost no tumors with alterations of the p18 gene have so far been identified, with the rare exception of several cases of multiple myeloma and childhood T lineage acute lymphoblastic leukemia (ALL).^{92,98–101} No rearrangements or deletions of the p19 gene were observed in hematologic malignancies including pediatric ALL with translocations involving chromosome 19p13.^{102–104}

Another group of CDKs includes p21^{Waf1} (also termed CIP1, SDI1 or CAP20),^{105–108} p27^{Kip1},^{109,110} and p57^{Kip2},^{111,112} which contain a homologous amino-terminal CDK inhibitory domain. The p21 family inhibits the activities of several cyclin/CDK complexes including cyclin D/CDK4, cyclin E/CDK2, and cyclin A/CDK2. p21 also inhibits the activity of the proliferating cell nuclear antigen (PCNA), a DNA replication factor.^{113,114} The inhibitory activities for the CDK family proteins and for PCNA reside in separate protein domains of p21.^{115,116} At least two pathways for the induction of p21 have been described: a p53-dependent pathway activated by DNA damage and a p53-independent pathway activated by mitogens at the entry into the cell cycle.^{105,106,117–119} The p27 is involved in blocking progression through the G1 phase in cells exposed to cyclic AMP¹²⁰ and TGF- β .¹²¹ Although the p21 family of proteins was originally thought to be exclusively an inhibitor of the cyclin/CDK, more recently it has been proposed as also acting as an assembly factor for the cyclin

Table 1 Inactivation of tumor suppressor genes in primary ATLL

Genes	Subtype	Frequency (%)		Sample	Details	Refs	
p15 ^{INK4B}	acute/lymphoma	4/23 (17)	#1	DNA	homozygous deletion	90	
	acute	23/77 (30)	#2	DNA	homozygous deletion	146	
	acute	7/34 (21)		DNA	homozygous deletion (exon 2)	147	
	acute/lymphoma	0/23 (0)		DNA	methylation	165	
	chronic	0/14 (0)	#1	DNA	no deletion and mutation	90	
	chronic	0/37 (0)	#2	DNA	homozygous deletion	146	
	chronic	3/23 (13)		DNA	homozygous deletion (exon 2)	147	
	chronic/smoldering	0/13 (0)		DNA	methylation	165	
	not described	5/21 (24)		DNA	homozygous deletion	148	
	not described	1/10 (10)		DNA	homozygous deletion	149	
	p16 ^{INK4A}	acute/lymphoma	4/23 (17)	#1	DNA	homozygous deletion	90
		acute/lymphoma	7/25 (28)		DNA	homozygous deletion and mutation	149
		acute	25/77 (33)	#2	DNA	homozygous deletion	146
acute		23/34 (68)		DNA	homozygous deletion	147	
acute		(24)		DNA	deletion	165	
acute		(47)		DNA	methylation	165	
lymphoma		(0)		DNA	deletion	165	
lymphoma		((73)		DNA	methylation	165	
chronic		1/14 (7)		DNA	homozygous deletion	90	
chronic/smoldering		1/19 (5)		DNA	homozygous deletion	149	
chronic		2/37 (5)		DNA	homozygous deletion	146	
chronic		(17)		DNA	methylation	165	
smoldering		(17)		DNA	methylation	165	
chronic		6/23 (26)		DNA	homozygous deletion	147	
not described		5/21 (24)		DNA	homozygous deletion	148	
not described		0/2 (0)		DNA	no deletion and mutation	143	
not described		1/10 (10)		DNA	homozygous deletion	150	
p18 ^{INK4C}		acute/lymphoma	0/23 (0)		DNA	no deletion and mutation	167
		chronic	0/16 (0)		DNA	no deletion and mutation	167
p19 ^{INK4D}		acute/lymphoma	0/28 (0)		DNA	no deletion and mutation	103
		chronic	0/16 (0)		DNA	no deletion and mutation	103
p21 ^{WAF1}		lymphoma	(3) ^a		protein	expression	unpublished
p27 ^{KIP1}		acute/lymphoma	1/24 (4)		DNA	mutation	202
	chronic	0/14 (0)		DNA	no deletion and mutation	202	
p53	acute/lymphoma	5/10 (50)		DNA	mutation	212	
	acute	2/6 (33)		DNA	mutation ^b	211	
	acute	1/12 (1)		DNA	mutation	213	
	lymphoma	1/4 (25)		DNA	mutation ^c	200	
	chronic	0/1 (0)		DNA	no mutation	212	
	chronic	0/6 (0)		DNA	no mutation	211	
	chronic/smoldering	0/4 (0)		DNA	no mutation	213	
	not described	3/10 (30)		DNA	mutation ^d	29	
	not described	0/14 (0)		DNA	no mutation	148	
	not described	1/5 (20)		DNA	mutation ^e	214	
	acute	0/6 (0)		mRNA	no altered expression ^b	211	
	chronic	0/6 (0)		mRNA	no altered expression	211	
	acute	4/5 (80)		mRNA	high expression	213	
	chronic	1/1 (100)		mRNA	high expression	213	
	acute	1/1 (100)		protein	overexpression ^b	211	
	lymphoma	2/4 (50)		protein	overexpression ^c	200	
	not described	3/3 (100)		protein	abnormal pattern ^d	29	
	not described	1/5 (20)		protein	overexpression ^e	214	
	Rb	acute/lymphoma	1/25 (4)		DNA	homozygous deletion	230
		chronic	1/15 (7)		DNA	homozygous deletion	230
		not described	5/10 (50)		protein	loss	148

^aMean of the frequency of p21^{WAF1} expressing cells for 12 samples detected by immunohistochemical staining.

^bThe same samples were analyzed for DNA, RNA and protein of p53. One sample has genomic mutation and overexpression of protein by immunohistochemical staining, but mRNA level was not altered by Northern blot analysis.

^cThe same samples were analyzed for DNA and protein alterations of p53. Among the samples overexpressing p53 protein by immunohistochemical staining, one had a detectable p53 mutation.

^dThe same samples were analyzed for DNA and protein alterations of p53. Immunohistochemical staining was performed for the samples with p53 mutation and revealed high expression in two and lack of expression in one.

^eThe same samples were analyzed for DNA, RNA and protein of p53. Among five samples, one sample had both mutation and overexpression of p53.

^fOne has p53 mutation.

All the samples #1 are included in the samples #2.

Table 2 Alterations of tumor suppressor genes in ATLL cell lines according to previously published reports

Cell line	Tax mRNA	p53 DNA/protein	p16 DNA/mRNA	p18 DNA/mRNA	p19 DNA/mRNA	p21 DNA/mRNA/protein	p27 DNA/mRNA	Rb DNA	Ref
ATL1K	+	WT/+++		WT/+					167, 213, 214
C10/MJ	+	WT/++				ND/+/+			34, 35, 185, 189, 220
C81-66-45		WT/ND	ND/+	ND/-	ND/±	ND/+/+	ND/+		69, 189
C91/PL		WT/ND				ND/+/+/+			189
DA202		WT/++				ND/+/+/+			185, 220
E55/PL		mut/ND				ND/+/+/+			189
ECI155		WT/+							220
FCL62		WT/+							220
Hayai				WT/-	WT/±				168
HUT102	+	WT/++	WT/+	WT/-	ND/+	ND/+/+	ND/+		69, 70, 167, 168, 185 34, 220, 214 189
LAF		WT/ND				ND/+/+/+			214
MF1	-	WT/+							214
MF3	-	WT/+							214
MJ		WT/ND				ND/+/+			189
MT1	± ^a	mut/+/+	WT/+	WT/-	WT/±				34, 70, 168, 213, 214
MT2	+	WT/++	WT/+	WT/++	WT/±	ND/+/+	ND/+		69, 70, 139, 167, 168, 185 34, 189, 213, 214, 220, 224
MT4	-		ND/-	WT/± ^b	ND/±	ND/+/+/ND	ND/±		35, 69, 70, 167, 168
N1185		WT/+				ND/+/+/+			185, 189, 220
N1186		WT/+				ND/+/+/+			185, 189, 220
NS1		WT/ND				ND/+/+/+			189
OCH			ND/+	ND/-	ND/+	ND/+/+/ND	ND/+		69
SH	ND ^c	loss/-							214
SLB1	+			WT/-					35, 167
SO4			homo ^d /ND	WT/ND	WT/ND		WT/ND	WT	90, 167, 230
Tsuka (ST1)			homo ^d /ND	WT/ND	WT/ND		WT/ND	WT	90, 167, 230

Blank, no information; ND, not described; WT, wild-type genomic DNA, mut, mutation; homo, homozygous deletion.

^aTAX protein is not expressed (Ref. 213).

^bExpression was detected by RT-PCR (Ref. 165), but not by Northern blot analysis (Ref. 166).

^cProtein was not detected (Ref. 307).

^dp15 was also homozygously deleted.

D/CDK4 complex.^{122,123} Structural abnormalities of the coding region of p21, p27 and p57 genes are very rare in human cancers.¹²⁴⁻¹²⁷

The p53 gene is a nuclear phosphoprotein which induces transcription of p21¹⁰⁵ and the DNA repair gene, GADD45^{128,129} in response to DNA damage. Careful analysis of the p53 gene has shown that it is frequently mutated in more than 50 varieties of human tumors including lung, breast, thyroid, gastrointestinal, ovarian and brain tumors as well as lymphomas/leukemias.¹³⁰⁻¹³⁵ These observations support the concept that p53 functions as a tumor suppressor gene.^{135,136}

Role of INK4 family in ATLL

The homozygous deletion of p16 gene has been detected at a very high rate in many types of tumor cells including leukemias,^{83,96,98,101,137,138} especially in T-ALL, in which it is deleted in about 70% of cases.^{92,139-145}

Several investigators including ourselves have reported alterations of the p16 occurring in 17-68% of the acute and 5-26% of the chronic ATLL samples and the p15 gene was structurally abnormal in 21-30% of the acute and 0-13% of the chronic ATLL specimens.^{90,146-150} In total, genomic alterations of the p16 gene occurred in 55 of 136 acute/lymphomatous (40%) cases and nine of 79 (11%)

chronic/smoldering ATLL samples. For the p15 gene, 30 of 111 (27%) acute/lymphomatous cases and three of 60 (5%) chronic/smoldering ATLL samples had alterations. Both genes were often simultaneously deleted, which is consistent with the proximity of the two genes on chromosome 9p21, being separated by only 25 kb.

Point mutations of the p16 gene are exceedingly rare. They have occasionally been reported in B cell-type non-Hodgkin's lymphoma (NHL),¹⁵¹ ALL^{141,144,145} and chronic lymphocytic leukemia (CLL).^{142,143} Missense mutations (codons 43 and 97) and a nonsense mutation (codon 72) of p16 were identified in two acute and one lymphomatous ATLL, respectively.¹⁴⁹ Thus, the p16 gene is rarely inactivated by point mutations, but lymphoid malignancies may be more frequent target when it occurs. All of the mutations involved the ankyrin repeats. These data and a mutational analysis done by us show⁷⁶ that the ankyrin repeats are critical to the function of the protein.

The association of p15 and p16 deletions with the development of acute/lymphomatous but not chronic/smoldering ATLL suggests that these genetic events may help drive the late stage of leukemogenesis in ATLL. In our study, an instructive case had no detectable deletion of the p15 and p16 genes during the chronic phase whereas both genes were homozygously deleted in the acute phase. A chronic ATLL patient with homozygous deletion of the p16 gene progressed to acute ATLL and died within 6 months, indicating that loss of p16 can precede the transformation from chronic to acute

ATLL.⁹⁰ Similarly, alteration of the p16 gene is involved in the progression to lymphoid blastic crisis from the chronic phase of chronic myelogenous leukemia (CML),^{152,153} as well as the evolution from low grade to high grade NHL.^{91,154} Furthermore, this is in agreement with the higher frequency of deletions of the p15 and p16 genes in high grade lymphoid malignancies, such as ALL, lymphoblastic or diffuse large cell lymphoma as compared to their low grade counterparts.^{155,156} Therefore, the development of deletions of the p15 and p16 genes help define a distinct clinical and pathological subgroup of hematologic neoplasms characterized as lymphoid origin, a pathologically high grade malignant behavior, progressive disease, and poor outcome. ATLL patients with deleted p15 and/or p16 genes have a significantly shorter survival than those with both genes preserved. None of the acute ATLL individuals who had either their p15 or p16 genes deleted, survived for 3 years, whereas those whose ATLL cells had these genes intact, survived up to 8 years.¹⁴⁶

Methylation of the 5' CpG island of p15 and p16 genes is associated with transcriptional silencing of these genes in many neoplasms including leukemias and lymphomas.^{157–164} The p16 gene is more frequently methylated in fresh tumor cells isolated from patients with acute (47%) and lymphomatous (73%) than in those with chronic (17%) and smoldering (17%) ATLL. The expression of p16 mRNA was markedly decreased in the samples with methylated p16 gene, especially if the promoter region was methylated. In contrast, methylation of p16 was not identified in asymptomatic carrier or uninfected individuals.¹⁶⁵ Tax-immortalized T cell lines and normal CD4⁺ T cells expressed comparable levels of p16 mRNA.⁶⁹ The methylation of the p15 gene in primary ATLL cells has not been reported in any types of ATLL. These data indicate that alterations of p16 may be more relevant to the progression of ATLL than those of p15.

We have shown that structural changes of the p18 gene rarely occur in over 100 samples of ATLL and NHL including primary samples and cell lines. PCR-SSCP analysis revealed an occasional polymorphism at codon 114 (GGC to GGT) which has been reported in other tumors.¹⁶⁶ Expression of p18 was often undetectable in HTLV-I-associated cell lines whereas it was expressed in normal CD4⁺ T cells, HTLV-I non-infected leukemia/lymphoma cell lines, and purified leukemic cells from HTLV-I uninfected acute leukemia patients.^{69,167–169} These results suggest that inactivation of p18 may be peculiar to ATLL. Further studies of expression of p18 in fresh ATLL cells will also be of interest.

Abnormalities of the p19 gene are apparently rare events in hematologic malignancies. Structural alterations of p19 were not detected in 45 primary ATLL samples (acute/lymphomatous and chronic) and two ATLL cell lines (SO-4 and ST-1) using Southern blot analysis and PCR-SSCP.¹⁰³ Expression of p19 mRNA was detected in both HTLV-I infected and non-infected T cell lines, but levels appeared to be slightly decreased in the HTLV-I infected T cell lines.^{69,168} Protein amounts of p18 and p19 in primary ATLL samples have not yet been reported.

Oncogenic effects of Tax may be due to its interaction with cellular proteins, consisting of several transcription factors including CREB, NF- κ B and SRF,^{170–173} and the family of I κ B transcriptional inhibitors.¹⁷⁴ I κ B family proteins contain 6–8 ankyrin motifs.¹⁷⁵ Tax binds to the ankyrin motifs of I κ B and inhibits the formation of the I κ B–NF- κ B complex, causing nuclear translocation of active NF- κ B.^{174,176} In a similar way, Tax binds to the four tandem repeats of the ankyrin motif in the p15 and p16 proteins and counteracts their inhibitory

activity, resulting in activation of CDK.^{70,168} Consistent with these observations, HTLV-I infected T cell clones and forced Tax-expressing cells developed resistance to p15-mediated growth arrest induced by TGF- β .¹⁶⁸ This phenomenon correlated with the inability of TGF- β to prevent hyperphosphorylation of pRb.¹⁷⁷ Also, Tax suppressed p16-mediated inhibition of U2OS cell growth.⁷⁰ The p15 and p16 can bind to Tax, but p18 and p19 cannot,¹⁶⁸ probably because the ankyrin motifs are only loosely conserved in the latter two CDKs. Similarly, Tax preferentially binds to NF- κ B-2 p100 as compared to NF- κ B-1 p105,¹⁷⁸ both of which have ankyrin motifs, suggesting Tax has some selectivity in its binding of ankyrin motifs.

Interestingly, a point mutation of p16 occurs in the HTLV-I infected MT-1 cells, and these cells do not express Tax. In contrast, wild-type p16 is abundantly expressed in MT-2 and HUT102 cells and they do express Tax. These results suggest that inactivation of p16 either by genetic alteration or possibly Tax expression is important for T cell immortalization.

The p18 gene is transcriptionally repressed by Tax through the E-box element between –710 and –510 of the p18 promoter.¹⁶⁸ This observation is consistent with recent reports on Tax-mediated trans-repression through the E-box element of the DNA polymerase β , lck and bax genes.^{36,179–181} However, p18 mRNA is detected in several tax expressing cell lines: ATL-1K (HTLV-I infected cell line) and the stably tax transfected Jurkat cell lines.¹⁶⁷ In addition, decrease of p18 mRNA expression is more obvious in HTLV-I infected than in Tax-immortalized T cells,⁶⁹ so Tax may not be the only determinant decreasing the expression of p18. Furthermore, while Tax repression may be important for the early carrier phase, it is probably not very relevant for the later phase of the disease when most of the HTLV-I infected cells no longer express Tax.⁴⁰

Role of p21 family in ATLL

The first identified CDKI was p21,^{71,72,74,182} which is a protein accumulating in both senescent human fibroblasts and a melanoma cell line during its differentiation.⁷⁴ In nontransformed cells with a diploid karyotype, the p21 is an abundant protein component that forms a quaternary complex with most of the cyclin/CDK complexes (cyclin A/CDK2, cyclin B/cdc2, cyclin E/CDK2 and cyclin D/CDK4) and PCNA.^{79,80} Transfection with a p21 cDNA in tumor cell lines resulted in suppression of their growth *in vitro*.⁷¹ Additional studies showed that over-expression of p21 inhibited proliferation of transformed cells *in vitro* as well as *in vivo*.^{183,184} A relationship between expression of p21 and apoptosis in a p53-independent manner was reported in HTLV-I-transformed lymphocytes after their exposure to adriamycin.¹⁸⁵ These observations suggested that p21 could be a tumor suppressor. However, mutations within the coding region of the p21 gene have almost never been detected in any type of malignancy including ATLL.^{90,186–188}

The p21 mRNA was expressed more abundantly in both IL-2-dependent and -independent HTLV-I infected T cell lines compared with normal T cells.⁶⁹ This phenomenon was observed irrespective of the functional status of p53.¹⁸⁹ At the protein level, the expression of the p21 was also elevated in HTLV-I infected T cell lines.¹⁸⁹ In contrast, p21 expression was almost undetectable in uninfected human T cell lines known to have mutated p53 genes (HUT78, Jurkat and CEM).^{69,189} The following observations support the hypothesis that Tax may be responsible for the high expression of p21.

First, T cell lines immortalized by tax express p21 mRNA at much higher levels than normal CD4⁺ T cells.^{69,189} Secondly, p21 expression is induced in Jurkat cells transfected with tax;¹⁸⁹ and thirdly, in related studies, transient transfection of tax was able to activate a reporter gene attached to the p21 promoter even in p53 null cells.¹⁸⁹ Surprisingly, however, deletion of the p53 response element in the p21 promoter correlated with a loss of Tax inducibility as measured by reporter gene study.¹⁸⁹ These findings suggest that the Tax protein is at least partially responsible for the p53-independent expression of p21 in HTLV-I infected cells; however, the p53 response element or a sequence in the vicinity of this site in the p21 promoter region is required for Tax transactivation.

The constitutive high level expression of p21 in HTLV-I infected T cell lines is paradoxical because these cells proliferate rapidly despite the presence of high levels of this protein. Other examples of this dysregulation are the high expression level of p21 in brain tumors¹⁹⁰ and acute myelogenous leukemia (AML) cells,¹⁹¹ but not in normal brain and bone marrow cells. Mutational inactivation of p21 has been shown not to be the case.^{124,188} In HTLV-I infected T cell lines, the anti-proliferative effect of p21 might be directly suppressed or alternative pathways might stimulate cell cycle progression regardless of the presence of a functional p21. Tax, like Sv40 T-antigen,⁷² c-Myc,¹⁹² or B-Myb,¹⁹³ might be able to cancel the anti-proliferative function of p21. Also, the elevated amounts of p21 produced in HTLV-I infected cell lines might still be under the level required to promote G1 arrest; recent data indicate the existence of a dose-dependent effect of p21 to inhibit cyclin-CDK complexes.⁸⁸ Alternatively, the cells could have an alteration in downstream target of p21. This has been reported in melanoma which develop a mutation of CDK4.^{194,195} In addition to being a potent inhibitor of most cyclin/CDK complexes,^{45,72,182,196,197} p21 also promotes the assembly of cyclin D/CDK4 complex.^{88,89} The high expression of p21 induced by Tax in HTLV-I infected T cells might, therefore, play a role in the proliferation of these transformed cells by acting as a factor that continuously promotes the assembly and activation of cyclin/CDK complexes. Furthermore, recent studies have raised the possibility that the p21 may function as a transcriptional coactivator for NF- κ B.¹⁹⁸ Elevated NF- κ B binding activity has been suggested to contribute to the oncogenic properties of Tax, possibly by disrupting the normal growth regulatory mechanisms of cellular genes.¹⁹⁹

Although *in vitro*, high levels of p21 protein occur in HTLV-I infected T cells and Tax immortalized T cell lines, most ATLL cells *in vivo* do not express p21 protein. In our study, only 3% of lymphomatous ATLL cells expressed p21 protein whereas 26% of the cells in other high grade lymphomas including Burkitt and lymphoblastic lymphomas expressed p21 protein by immunohistochemical staining (unpublished data, manuscript in preparation). Furthermore, Mansukhani *et al*²⁰⁰ also revealed that only one of four ATLL cases showed p21 immunoreactivity in more than 10% of tumor cell nuclei. Levels of p21 protein are low in primary ATLL cells probably because Tax is not expressed in these cells. Another possible explanation is that p21 may be rapidly degraded as reported for p27 protein in colorectal carcinomas; the latter protein is degraded by increased proteasome activity in the tumor cells.²⁰¹

We found only two cases of p27 alterations among 42 ATLL samples using Southern blot and PCR-SSCP analyses; a homozygous deletion and a point mutation.²⁰² Thus, development of ATLL is usually not the result of a structural alteration of p27. The p27 mRNA was detected in both HTLV-I infected

and non-infected T cell lines, however, the level was slightly higher in HTLV-I non-infected T cell lines.⁶⁹ To date, the protein level of p27 in ATLL has not been reported.

The p57 is transcriptionally repressed in Wilms' tumor²⁰³ and CML during blast crisis¹²⁶ but mutations of the p57 gene have not been detected in any neoplasm.^{126,127} A causative role of p57 in the development of ATLL is not clear at this time.

Role of p53 in ATLL

p53 is a well characterized tumor suppressor gene on chromosome 17p. It is implicated in cell cycle regulation through induction of p21. While p53 is also known to have a role in apoptosis,^{204,205} the mechanisms by which p53 induces apoptosis are still largely unknown. Inactivation of the p53 gene contributes not only to dysregulation of the cell cycle machinery, but also to defects in the DNA repair system and to cellular immortalization.²⁰⁶ The p53 gene is frequently mutated in a variety of tumors including hematologic malignancies.^{132,133,207,208} Unlike functional p53, which is rapidly degraded within 5 to 20 min after its synthesis,²⁰⁹ mutant versions of the proteins have a half-life of 4 to 8 h²¹⁰ and accumulate in the cells, thus becoming immunohistochemically detectable.

At most, 50% of individuals have p53 mutations in primary ATLL cells.^{30,211-214} In our series, leukemic cells of five of 10 acute/lymphomatous ATLL cases had homozygous p53 mutations. One informative case had no detectable p53 mutation in the ATLL cells during the chronic phase of the disease, but had a homozygous point mutation when he progressed to acute ATLL.²¹² Our results were mirrored in those of other investigators;^{211,213} and taken together, eight of 28 (29%) acute/lymphomatous and none of 11 (0%) chronic/smoldering ATLL samples were found to have mutations of p53. These data suggest that inactivation of the p53 gene is frequent and associated with the transition to a more aggressive leukemic phenotype. Similarly, in other lymphomas, p53 mutations also occur as relatively late events in tumorigenesis.^{215,216} In cell lines, mutations of the p53 gene can be found only in a minority of HTLV-I-transformed cell lines.

We have previously shown that the several CCGG tetranucleotides throughout the coding region of the p53 gene are fully methylated in HTLV-I-transformed cells.²¹⁷ Endogenous methylation of cytosines and transition of these methylated cytosines to thymidines by deamination is considered as one of the mechanisms of p53 mutations.²¹⁸ Repression of DNA polymerase β , a DNA-repair enzyme, in HTLV-I-immortalized cells³⁶ may also cause genetic alterations including mutations of the p53 gene in ATLL.

The transcription level of p53 in ATLL was higher than that of normal PBMNC regardless of the mutational status of p53.²¹³ For cell lines, expression of p53 mRNA by Northern blot hybridization in MT-1 and ATL-1K (both with a p53 mutation) and MT-2 (wild-type p53) cell lines was also high compared with that of normal PBMNC.²¹³

The protein level of p53 in primary ATLL samples is not conclusive from the data available. Several investigators reported that the samples with high expression of p53 protein had missense mutations.^{211,214} This contrasts with the finding that some ATLL cases with p53 mutations lack detectable p53 protein.^{29,200} Furthermore, several cases having wild-type p53 highly express this protein.²⁰⁰ In cell lines, elevated steady-

state levels of p53 protein were demonstrated in both HTLV-I-transformed cell lines having a wild-type sequence of the p53 coding region and those with a mutated version of the gene (MT-1 and HUT-102).^{189,214,219,220} Thus, wild-type as well as mutant p53 are stabilized post-translationally in HTLV-I infected T cell lines, probably due to prolongation of protein half-life.²¹⁹ We have found that the half-life of p53 was 2 h in HTLV-I-transformed cells,²¹⁷ which was about six times that of human wild-type p53.²²¹ Low expressors of p53 protein do not have mutations of the gene in HTLV-I infected cell lines.²¹⁴

In one study, Tax expression was found in ATLL samples expressing high levels of wild-type p53 protein, but it was not detectable in cells that either expressed low levels of p53 or had a mutation of the gene. The levels of p53 mRNA were similar among the samples regardless of p53 protein levels.²¹⁴ Post-translational mechanisms other than missense mutation would thus appear to stabilize p53 in the Tax-expressing cells but not in those containing undetectable levels of Tax. Alternatively, the co-expression of Tax and high levels of p53 may be epiphenomenon.

Some experimental evidence suggests that the function of wild-type p53 in HTLV-I-transformed cells appears to be impaired. Some data concern the lack of appropriate p53-mediated response to ionizing radiation. Hematopoietic cells with functional p53 undergo apoptosis after ionizing radiation;^{222,223} however, apoptosis was not induced in either E55/PL (p53 mutation+) or MJ (p53 mutation-) HTLV-I infected T cells.¹⁸⁹ These observations are consistent with other reports that p53-responsive elements are not appropriately upregulated in HTLV-I infected T cells, particularly in IL-2-independent cell lines.²²⁰ In another study, luciferase assays with a reporter plasmid containing p53-binding sites revealed an impairment in the transactivating function of p53 in the Tax-expressing T cells, even though the cells had wild-type conformation of the p53 gene.²²⁴

An additional suggestion of p53 dysfunction in HTLV-I infected cells was shown by a weak induction of GADD45 expression after γ -irradiation. A functional p53 normally mediates a robust expression of GADD45 in response to DNA damage.^{128,129} For example, normal T cells responded to γ -irradiation with a mark increase of expression of the GADD45 gene. In contrast, under the same conditions, minimal enhancement of GADD45 transcription was observed in HTLV-I-infected cell lines and a Tax-transformed cell line.¹⁸⁹

Functional impairment of p53 is associated with several DNA transforming viruses. Adenovirus, SV40, and human papillomavirus encode proteins that either bind and functionally inactivate p53 (E1B of adenovirus and T antigen of SV40), or degrade the p53 protein (E6 protein of papilloma virus).²²⁵ The mechanism of p53 impairment in HTLV-I infected T cells could be due to interactions with a viral protein or a virally induced cellular protein. However, no complex formation between p53 and Tax has been observed,^{214,219,220,226} nor does Rex, another HTLV-I virus specific regulatory protein, complex with p53.²²⁰ Moreover, Tax does not directly induce changes of p53, since early passage of Tax-transduced primary T cells does not show aberrant expression or function of p53; it is observed only in fully immortalized cells. Dysfunction of p53 is probably a rather late event in the Tax-induced immortalization process.²²⁴ In fact, one series of experiments suggested that impaired p53 function was linked to progression from IL-2-dependent to IL-2-independent growth in HTLV-I-transformed lymphocytes. Levels of p53 protein were consistently higher in IL-2-inde-

pendent HTLV-I-transformed cell lines compared with IL-2-dependent ones. Furthermore, reporter gene studies using a p53 response element suggested that a dysfunctional p53 was observed only in IL-2-independent cell lines.²²⁰

A new member of the p53 gene family, p73, has been recently isolated.²²⁷ Overexpression of p73 can induce expression of p21 and cause apoptosis in the p53-negative osteosarcoma cell line, SAOS-2.²²⁸ Thus, p73 could be a new tumor suppressor. No structural alterations of p73 were identified in over 100 samples from leukemia and lymphoma cell lines and patient samples. Gene expression of p73 was not detectable in eight of 21 T-ALL/lymphoblastic lymphoma (LBL) cell lines and three of eight T-ALL/LBL patient samples. However, only one ATLL cell line, HUT-102, was studied and found to express p73.²²⁹ The role of p73 in the development of ATLL is an important issue to be addressed by analyzing a large number of ATLL samples.

Role of Rb in ATLL

We looked for structural alterations of the entire Rb gene including the promoter region in 40 primary ATLL and two ATLL cell lines (SO-4 and ST-1) using Southern blot and PCR-SSCP analyses. Homozygous deletions of exon 1 were found in two primary cases for an incidence of 5% (one of 21 acute, none of four lymphomatous, and one of 15 chronic ATLL).²³⁰ Hangaishi *et al*¹⁴⁸ also reported that structural alterations of the Rb gene were not found in 19 ATLL cases by Southern blot analysis. However, in the same series, five of 10 ATLL samples (50%) had either undetectable or remarkably low levels of expression of the Rb protein.¹⁴⁸ Hypermethylation of the promoter region of Rb has been associated with decreased expression of the Rb gene in retinoblastomas²³¹⁻²³³ and, thus, a similar phenomenon may occur in ATLL.

Several investigators have proposed that inactivation of the Rb locus may contribute to progression rather than to initiation of many solid tumors.^{234,235} However, less is known concerning hematologic malignancies. The frequency of either low or non-detectable expression of Rb in CLL does not change with either clinical progression or time from diagnosis, suggesting that loss of Rb expression is more closely associated with the development rather than the progression of CLL.^{236,237} In our study, because the frequency of Rb deletion was similar in acute ATLL (1/21) and chronic ATLL (1/15), loss of the Rb gene is probably not associated with progression of the disease. Future studies should measure the level of Rb protein in different stages of ATLL.

An inverse correlation occurs between functional integrity of p16 and Rb in many tumor cell lines and primary tumors; cells with a p16 deletion often have a wild-type pRb and vice versa.^{106,116,118,238-240} Moreover, forced over-expression of wild-type p16 induced G1 arrest in pRb-positive cells but had no effect in pRb-negative cells.^{94,99-102} The case with p53 is less clear, with some studies showing²⁴¹⁻²⁴³ and other reports not finding^{106,148} an inverse correlation between a tumor having a p53 mutation and having normal p16 and Rb genes. The study of DNA-transforming viruses would lead to the prediction that the elimination of both p53 and Rb is more transforming than the inhibition of either one alone. The papilloma virus produces E6 (inactivates p53) and E7 (inhibits Rb); the adenovirus synthesizes E1A (inhibits Rb) and E1B (inactivates p53); and SV40 makes the large T antigen (inhibits both Rb and p53). Furthermore, mice deficient in both p53 and Rb showed a faster rate of tumor growth and a shorter survival

rate than mice deficient in either the p53 or Rb gene.²⁴⁴ Thus, a growth advantage may result from both being inactivated in the same cells.

Aberrations of CDKs, Rb, and p53 in ATLL as examined by Hangaishi *et al*¹⁴⁸ and ourselves^{90,103,167,202,230} are summarized in Table 3. Because of the short distance between p15 and p16, both genes are often co-deleted and can be thought of as a group. Notably, the ATLL samples and cell lines had either a CDKI, Rb or p53 abnormality; however, except for one case, none of these samples had more than one alteration of these three families of cell cycle-related proteins. Taken together, the results emphasize that the CDKs, Rb and p53 proteins are working on a common pathway of controlling the S phase of the cell cycle. Inactivation of one part of this pathway possibly alleviates the need for inactivation of other genes in the development of ATLL.

Role of DNA mismatch repair genes

Microsatellites are short tracts of (CA)_n nucleotide repeats existing throughout the genome. Microsatellite instability (MSI) initially reported in hereditary nonpolyposis colorectal cancer (HNPCC)²⁴⁵ represents either an expansion or reduction of these sequences. This phenomenon appears to reflect multiple replication errors²⁴⁶ owing to germ line mutations of mismatch repair genes including human MutL homologue 1 (hMLH1),^{247,248} human MutS homologue 2 (hMSH2),^{249,250} human post-meiotic segregation 1 (hPMS1) and 2 (hPMS2),^{251,252} and GT-binding protein (GTBP).²⁵³ Several studies have shown that mice engineered to be deficient in MLH1, MSH2 or PMS2 had MSI.^{254–257} MSI has been observed not only in solid tumors, but also in hematologic malignancies such as blastic crisis of CML,²⁵⁸ AML,²⁵⁹

aggressive lymphoma in HIV infected patients²⁶⁰ and ALL.²⁶¹ We have shown that about 40% of ATLL samples had MSI as the individuals evolved into their acute/lymphomatous phase.²⁶² This frequency was higher than in other hematologic malignancies. One possibility is that MSI in ATLL is associated with the progression rather than the initiation of the disease, similar to what occurs in colon and gastric cancers^{263–268} and the blastic crisis of CML.²⁵⁸

The predisposition to lymphoid tumors was reported in MLH1-, MSH2- and PMS2-deficient mice.^{255,256,269} Development of lymphoid tumors such as ALL, CLL, and malignant lymphomas was also reported in human HNPCC kindreds.^{270–273} These observations imply that defects in the mismatch repair apparatus may cause accumulation of mutations in key oncogenes or tumor suppressor genes as well as in microsatellite sequences, thus contributing to the development of tumors.^{250,251,274} However, the proportion and spectrum of sporadic cancers associated with mutations in mismatch repair genes has not been clearly elucidated at this time. Furthermore, the relationship between mutations of mismatch repair genes and MSI have not been observed clearly in sporadic tumors of various tissue with several exceptions.^{266,275–280}

We looked for genetic alterations of the hMSH2 gene in 41 ATLL samples and two ATLL cell lines using polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) technique. Six ATLL samples showed two types of base changes of hMSH2, which probably represent polymorphisms.²⁸⁰ Also, to date, mutations of the hMSH2 gene have not been detected in other hematologic malignancies.²⁸¹

Hangaishi *et al*²⁸² found the mutations of the hMLH1 gene in lymphoid leukemia cell lines only. However, a HTLV-I infected cell line, MT-1, was found to have a wild-type hMLH1 gene.²⁷⁹ Germ line mutations in hMSH3 have not been found in a variety of neoplasms including ATLL.^{279,283,284}

Table 3 Aberrations of CDKs, Rb, and p53 in ATLL

Type	p15 ^{INK4B}	p16 ^{INK4A}	p18 ^{INK4C}	p19 ^{INK4D}	p27 ^{KIP1}	Rb gene	Rb protein	p53	Ref
Acute	hemi	hemi	WT	WT	WT	homo	N	N	90, 103, 167, 202, 230
Acute	homo	homo	WT	WT	WT	WT	N	N	90, 103, 167, 202, 230
Acute	hemi	hemi	WT	WT	WT	WT	N	N	90, 103, 167, 202, 230
Acute	hemi	hemi	WT	WT	WT	WT	N	N	90, 103, 167, 202, 230
Acute	homo	homo	WT	WT	WT	WT	N	N	90, 103, 167, 202, 230
Acute	homo	homo	WT	WT	WT	WT	N	N	90, 103, 167, 202, 230
Acute	WT	hemi	WT	WT	WT	WT	N	N	90, 103, 167, 202, 230
Lymphoma	WT	WT	WT	WT	homo	WT	N	N	90, 103, 167, 202, 230
Lymphoma	homo	homo	WT	WT	WT	WT	N	N	90, 103, 167, 202, 230
Lymphoma	WT	WT	WT	WT	mut	WT	N	N	90, 103, 167, 202, 230
Chronic	WT	hemi	WT	WT	WT	WT	N	N	90, 103, 167, 202, 230
Chronic	WT	WT	WT	WT	WT	homo	N	N	90, 103, 167, 202, 230
Chronic	WT	homo	WT	WT	WT	WT	N	N	90, 103, 167, 202, 230
Cell Line (TSUKA)	WT	homo	WT	WT	WT	WT	N	N	90, 103, 167, 202, 230
Cell Line (SO4)	WT	homo	WT	WT	WT	WT	N	N	90, 103, 167, 202, 230
Unknown	homo	homo	N	N	N	WT	loss	WT	148
Unknown	homo	homo	N	N	N	WT	WT	WT	148
Unknown	homo	homo	N	N	N	WT	WT	WT	148
Unknown	homo	homo	N	N	N	WT	N	N	148
Unknown	hemi	hemi	N	N	N	WT	N	N	148
Unknown	WT	WT	N	N	N	WT	loss	WT	148
Unknown	WT	WT	N	N	N	WT	loss	WT	148
Unknown	WT	WT	N	N	N	WT	loss	WT	148
Unknown	WT	WT	N	N	N	WT	loss	WT	148

homo, homozygous deletion; hemi, hemizygous deletion; WT, wild type; N, not tested or described; mut, mutation; loss, loss of Rb protein.

Studies have not yet expanded to the expression and methylation status of mismatch repair genes in ATLL although lack of expression of hMLH1 owing to cytosine methylation of the hMLH1 promoter region has been observed in sporadic colon tumors as well as colon and endometrial tumor cell lines.²⁷⁹

Somatic frameshift mutations of coding repeats within the TGF- β R2, Bax, IGF1R, hMSH3, and E2F4 (one of E2F family) genes are recurrent targets of MSI in many kinds of cancers. We have identified frameshift mutations at a multiple trinucleotide AGC (serine) repeat within the coding exon of the E2F4 gene in two of 10 (20%) acute/lymphomatous ATLL samples having MSI, suggesting that mutations of the E2F4 gene, presumably caused by an abnormality of one of the DNA repair genes, may participate in the progression of ATLL. No mutations were found in the single nucleotide repeat sequences within the TGF- β R2, Bax, IGF1R, and hMSH3 genes.²⁸⁴

Besides the mismatch repair proteins, other genes of DNA replication and repair system should be considered as possible mutational targets including those coding for helicase (BLM, WRN, XH2), DNA polymerase, double-strand break repair protein (RAD family), and nucleotide excision repair protein (XP, PCNA). In fact, the activity of DNA polymerase is low in HTLV-I-immortalized cells.³⁶

Other candidate tumor suppressor genes in ATLL

From several studies, chromosome 6q appears to be involved in the pathogenesis of a number of solid tumors.^{285–287} In hematologic malignancies, deletions involving the long arm of chromosome 6 are observed primarily in lymphoid malignancies, ie ALL,^{288–290} lymphoproliferative disorders and NHL.^{288,291–294} These results indicate that the long arm of chromosome 6 harbors a yet uncharacterized tumor suppressor gene(s) that is altered in many tumors. Cytogenetic studies showed that chromosome 6q is also often affected in acute/lymphomatous ATLL.^{295,296} We have identified a 4 cM commonly deleted region on chromosome 6q15–21 in samples of acute/lymphomatous ATLL by analysis for loss of heterozygosity.^{297,298} The evolution of chronic/smoldering to acute/lymphomatous ATLL may be fostered by inactivation of a putative tumor suppressor gene on the locus.

The DPC 4/SMAD 4 gene was identified as a candidate tumor suppressor gene at 18q21, with inactivation playing a possible role in the genesis of pancreatic cancers.²⁹⁹ We examined the structural status of the DPC 4/SMAD 4 gene using Southern blot and PCR-SSCP analyses for 43 ATLL samples as well as several other types of tumors. None of the primary tumor samples had a detectable abnormality, indicating that alterations of the DPC 4/SMAD 4 gene, unlike pancreatic cancer, are not associated with the occurrence of ATLL.³⁰⁰

Possible hypothesis regarding the development of ATLL

Our understanding of the pathogenesis of ATLL is evolving. A hypothesis concerning the development of ATLL can be stated as shown in Figure 1. HTLV-I infection causes acute expression of Tax, which can stimulate lymphocyte proliferation in the initial stage of ATLL. Tax not only regulates the expression of HTLV-I genes, but also activates the transcription of specific cellular genes including NF- κ B and inactivates I κ B. Increased level of p21 induced by Tax may be involved in the early stage of the disease through activation of NF- κ B

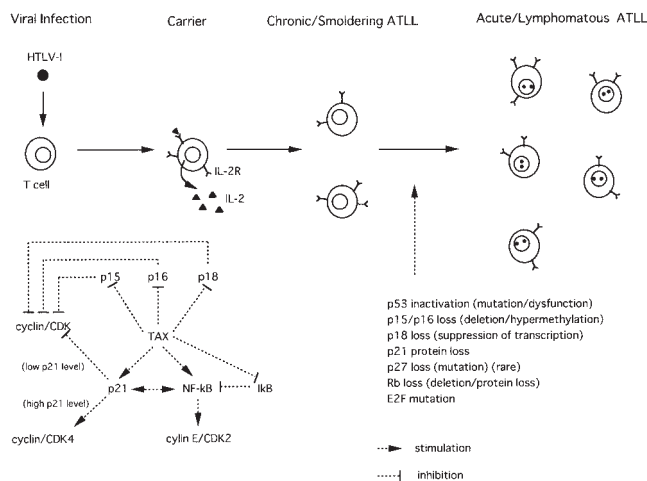


Figure 1 Schematic representation of the development of ATLL.

and assembly of cyclin/CDK complex. Tax transcriptionally represses p18 and functionally inactivates p15/p16 by binding them. Hyperexpression of IL-2 and IL-4 receptors induced by Tax in virally infected lymphocytes^{301,302} perhaps results in autocrine growth stimulation through production of IL-2 and other lymphokines.^{27,303} Tax can upregulate a number of cellular genes including proto-oncogenes (c-fos, c-jun and c-myc) and immediate-early genes (erg-1 and -2) in addition to growth factors. All of these Tax-related phenomena promote cell growth and cause a polyclonal or oligoclonal increase of the HTLV-I infected T lymphocytes. *In vitro*, HTLV-I infected T cells evolve from IL-2-dependent to IL-2-independent, suggesting that their growth is not maintained by an autocrine/paracrine mechanism. Functional inactivation of p53 protein might contribute to the abrogation of IL-2-dependent growth in HTLV-I infected T cells. Also, the Jak-STAT pathway may be activated constitutively in IL-2-independent HTLV-I-transformed T cells.³⁰⁴ While a link between this pathway and that of p53 is not clear at present, they may both be important in the acquisition of IL-2 independence.

The polyclonal and then oligoclonal expanded population of the HTLV-I infected T cells may have a slight growth advantage over normal T cells. After these initial stages, expression of Tax disappears followed by disappearance of p21. Evolution from chronic to acute ATLL may result from additional inactivations of tumor suppressor genes such as p53, p15, p16, p21, and Rb. Also, a low p21 level itself enhances cell growth because p21 promotes the assembly of active cyclin/CDK complexes at low concentrations whereas p21 inhibits activity of the complexes at higher concentrations.⁸⁹ If our thesis is accurate, ATLL will provide a valuable model for analyzing the role of tumor suppressor genes and multiple genetic events in the clonal development of cancer.

Gene therapy and other therapeutic approaches for ATLL

The therapeutic restoration of expression of wild-type tumor suppressor genes in ATLL might eventually involve gene therapy. In several model systems, features of the tumor phenotype can be suppressed *in vitro* through the restoration of expression of a tumor suppressor gene such as p53 and Rb. Highly efficient and targeted gene delivery vectors must be developed to be clinically useful.

Immunotherapeutic approaches may also be possible. If the

epitopes of mutant tumor suppressor genes were displayed on the malignant cells and could be presented to T lymphocytes by class I major histocompatibility complex molecules on the cell membrane, a cytotoxic immune response against these tumor cells might occur.³⁰⁵ A murine model has shown that a cytotoxic immune response can occur against cells expressing mutant p53.³⁰⁶ In addition, autoantibodies to p53 have been identified in some cancer patients although no evidence exists that these antibodies are directed against mutant p53 or that these individuals have an improved clinical response to their cancer.^{305–307}

Finally, novel chemotherapy and/or irradiation treatment schedules can be given to individuals with ATLL. DNA-injured cells with a p53 mutation do not stop at the G1 phase of the cell cycle for DNA repair before beginning DNA synthesis. This contrasts to cells with wild-type p53, which enforces a pause at the G1 phase of cell cycle after DNA damage. Therefore, the difference in the cell cycle control after DNA damage between malignant cells having an inactivated p53 and normal cells expressing wild-type p53 can be utilized to optimize chemotherapy and/or irradiation treatment.

Conclusion

Compelling evidence supports a pivotal role for altered tumor suppressor genes involved in leukemogenesis and lymphomagenesis of ATLL as well as progression of the disease from the chronic/smoldering to the acute/lymphomatous type. Disorder of cell cycle control exists in HTLV-I infected T cells during the transformation mediated by Tax. Further investigations are needed as to how the alteration of cell cycle regulatory genes are involved in the aberrant growth of HTLV-I infected T cells. Recent technical development of DNA microarray analysis will clarify the master gene(s)/molecule(s) for development and progression of ATLL. The mutant tumor suppressor genes may also provide a clue for innovative therapeutic approaches to treat ATLL.

Acknowledgements

We are indebted to our colleagues for continuous support and advice; to Kim Burgin and Marge Jacobs (posthumously) for their excellent secretarial help. Supported in part by the NIH grants, the Ko-So Foundation, Lymphoma Foundation of America, The Begell Foundation and the C and H Koeffler fund. HP Koeffler holds the endowed Mark Goodson Chair of Oncology research at Cedars-Sinai Medical Center/UCLA School of Medicine and is a member of the Jonsson Cancer Center.

References

- Uchiyama T, Yodoi J, Sagawa K, Takatsuki K, Uchino H. Adult T-cell leukemia: clinical and hematologic features of 16 cases. *Blood* 1977; **50**: 481–492.
- Hattori T, Uchiyama T, Tobinai T, Takatsuki T, Uchino H. Surface phenotype of Japanese ATL cells characterized by MoAbs. *Blood* 1981; **58**: 645–647.
- Yamada Y. Phenotypic and functional analysis of leukemic cells from 16 patients with adult T-cell leukemia/lymphoma. *Blood* 1983; **61**: 192–199.
- Yamada Y, Ichimaru M, Shiku H. Adult T cell leukaemia cells are of CD4+ CDw29+ T cell origin and secrete a B-cell differentiation factor. *Br J Haematol* 1989; **72**: 370–377.
- Poiesz BJ, Russetto FW, Gadar AF, Bunn PA, Minna JD, Gallo RC. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc Natl Acad Sci USA* 1980; **77**: 7415–7419.
- Miyoshi I, Kubonishi T, Yoshimoto S, Akagi I, Ohtsuki Y, Shiraishi Y, Nagata K, Hinuma Y. Type C virus particles in a cord T-cell line derived by co-cultivation of normal human cord leukocytes and human leukemic T-cells. *Nature* 1981; **294**: 770–771.
- Yoshida M, Miyoshi I, Hinuma Y. Isolation and characterization of retrovirus from cell lines of human adult T-cell leukemia and its implication in the disease. *Proc Natl Acad Sci USA* 1982; **79**: 2031–2035.
- Popovic M, Sarin PS, Robert-Guroff M, Kalyanaram VS, Mann D, Minowada J, Gallo RC. Isolation and transmission of human retrovirus (human T-cell leukemia virus). *Science* 1983; **219**: 856–859.
- Levine PH, Cleghorn F, Manns A, Jaffe ES, Navarro-Roman L, Blattner WA, Hanchard B, De Oliveira MS, Matutes E, Catovsky D, Shimoyama M, Tajima K, Sonoda S, Yamaguchi K, Takatsuki K. Adult T-cell leukemia/lymphoma: a working point-score classification for epidemiological studies. *Int J Cancer* 1994; **59**: 491–493.
- Blattner WA, Kalyanaram VS, Robert-Guroff M, Listner TA, Galton DA, Sarin PS, Crawford MH, Catovsky D, Greaves M, Gallo RC. The human type-C retrovirus, HTLV, in blacks from Caribbean region, and the relationship to adult T-cell leukemia/lymphoma. *Int J Cancer* 1982; **30**: 257–264.
- Hinuma Y, Komoda H, Chosa T, Kondo T, Kohakura M, Takenaka T, Kikuchi M, Ichimari M, Yunoki K, Sato I, Matsuo R, Takiuchi Y, Uchino Y, Hanaoka M. Antibodies to adult T-cell leukemia-virus-associated antigen (ATLA) in sera from patients with ATL and controls in Japan: a nation-wide sero-epidemiologic study. *Int J Cancer* 1982; **29**: 631–635.
- Saxinger W, Blattner WA, Levine PH, Clark J, Biggar R, Hoh M, Moghissi J, Jacobs P, Wilson L, Jacobson R, Crookes R, Strong M, Ansari AA, Dean AG, Nkrumah FK, Mourali N, Gallo RC. Human T-cell leukemia virus (HTLV-I) antibodies in Africa. *Science* 1984; **225**: 1473–1476.
- Robert-Guroff M, Weiss SH, Giron JA. Prevalence of antibodies to HTLV-I, II, and III in intravenous drug abusers from an AIDS endemic region. *JAMA* 1986; **255**: 3133–3137.
- Meytes D, Schochat B, Lee H, Nadel G, Sidi Y, Cerney M, Swanson P, Shaklani M, Kilim Y, Elgat M, Chin E, Danon Y, Rosenblatt JD. Serological and molecular survey for HTLV-I infection in a high-risk Middle Eastern group. *Lancet* 1990; **336**: 1533–1535.
- Shimoyama M, members of the Lymphoma Study Group (1984–87). Diagnostic criteria and classification of clinical subtypes of adult T-cell leukemia-lymphoma. *Br J Haematol* 1991; **79**: 428–437.
- Yamada Y, Tomonaga M, Fukuda H, Hanada S, Utsunomiya A, Tara A, Sano M, Ikeda S, Takatsuki K, Kozuru M, Araki K, Kawano F, Niimi M, Tobinai K, Hotta T, Shimoyama M. A new G-CSF-supported combination chemotherapy: LSG15, for adult T-cell leukaemia-lymphoma: Japan clinical oncology group study 9303. *Br J Haematol* 2001; **113**: 375–382.
- Bunn PA Jr, Schechter GP, Jaffe E, Blayney D, Young RC, Matthews MJ, Blattner W, Brodros S, Robert-Guroff M, Gallo RC. Clinical course of retrovirus-associated adult T-cell lymphoma in the United States. *N Engl J Med* 1983; **309**: 257–264.
- Catovski D, Greaves MF, Rose M, Galton DA, Goolden AWG, McCluskey DR, White JM, Lampert I, Bourikas G, Ireland R, Brownell AI, Bridges JM, Blattner WA, Gallo RC. Adult T-cell lymphoma-leukemia in Blacks from the West Indies. *Lancet* 1982; **1**: 639–643.
- Kinoshita K, Kamihira S, Ikeda S, Yamada Y, Muta K, Kitamura T, Ichimaru M, Matsuo T. Clinical, hematologic, and pathologic features of leukemic T-cell lymphoma. *Cancer* 1982; **50**: 1554–1562.
- Gessain A, Barin F, Vernant JC, Gout O, Maurs L, Calender A, de The G. Antibodies to human T-lymphotropic virus type-I in patients with tropical spastic paraparesis. *Lancet* 1985; **2**: 407–410.
- Osame M, Usuku K, Izumo S, Ijichi N, Amitani H, Igata A, Matsu-

- moto M, Tara M. HTLV-I associated myelopathy, a new clinical entity. *Lancet* 1986; **1**: 1031–1032.
- 22 Sugimoto M, Nakashima H, Watanabe S, Uyama T, Tanaka F, Ando M, Araki S, Kawasaki S. T-lymphocyte alveolitis in HTLV-I-associated myelopathy. *Lancet* 1987; **2**: 1220.
- 23 Nishioka K, Maruyama I, Sato K, Kitajima I, Nakajima Y, Osame M. Chronic inflammatory arthropathy associated with HTLV-I. *Lancet* 1989; **1**: 441.
- 24 LaGrenade L, Hanchard B, Fletcher V, Cranston B, Blattner W. Infective dermatitis of Jamaican children: a marker for HTLV-I infection. *Lancet* 1990; **336**: 1345–1347.
- 25 Mochizuki M, Watanabe T, Yamaguchi K, Takatsuki K, Yoshimura K, Shiroo M, Nakashima S, Mori S, Araki S, Miyata N. HTLV-I uveitis: a distinct clinical entity caused by HTLV-I. *Jpn J Cancer Res* 1992; **83**: 236–239.
- 26 Seiki M, Hattori S, Yoshida M. Human adult T-cell leukemia virus: molecular cloning of the provirus DNA and the unique terminal structure. *Proc Natl Acad Sci USA* 1982; **79**: 6899–6902.
- 27 Seiki M, Hattori S, Hirayama Y, Yoshida M. Human adult T-cell leukemia virus: complete nucleotide sequence of the provirus genome integrated in leukemia cell DNA. *Proc Natl Acad Sci USA* 1983; **80**: 3618–3622.
- 28 Wong-Staal F, Gallo RC. Human T-lymphotropic retroviruses. *Nature* 1985; **317**: 395–403.
- 29 Cesarman E, Chadburn A, Inghirami G, Gaidano G, Knowles DM. Structural and functional analysis of oncogenes and tumor suppressor genes in adult T-cell leukemia/lymphoma shows frequent p53 mutations. *Blood* 1992; **80**: 3205–3216.
- 30 Kiyokawa T, Seiki M, Imagawa K, Shimizu F, Yoshida M. Identification of a protein (p40x) encoded by a unique sequence pX of a human T-cell leukemia virus type I. *Gann* 1984; **75**: 747–751.
- 31 Nerenberg M, Hinrichs SH, Reynolds RK, Khoury G, Jay G. The tax gene of human T lymphotropic virus type I induces mesenchymal tumors in transgenic mice. *Science* 1987; **237**: 1324–1329.
- 32 Grassmann R, Berchtold S, Radant I, Alt M, Fleckenstein B, Sodroski JG, Haseltine WA, Ramstedt U. Role of human T-cell leukemia virus type 1 X region proteins in immortalization of primary human lymphocytes in culture. *J Virol* 1992; **66**: 4570–4575.
- 33 Grossman WJ, Kimata JT, Wong FH, Zutter M, Ley TJ, Ratner L. Development of leukemia in mice transgenic for the tax gene of human T-cell leukemia virus type I. *Proc Natl Acad Sci USA* 1995; **92**: 1057–1061.
- 34 Mori N, Murakami S, Oda S, Prager D, Eto S. Production of interleukin 8 in adult T-cell leukemia cells: possible transactivation of the interleukin 8 gene by human T-cell leukemia virus type I tax. *Cancer Res* 1995; **55**: 3592–3597.
- 35 Mori N, Gill PS, Mougil T, Murakami S, Eto S, Prager D. Interleukin-10 gene expression in adult T-cell leukemia. *Blood* 1996; **88**: 1035–1045.
- 36 Jeang KT, Widen SG, Semmes OJ 4th, Wilson SH. HTLV-I transacting protein, tax, is a trans-repressor of the human β -polymerase gene. *Science* 1990; **247**: 1082–1084.
- 37 Seiki M, Eddy R, Shows TB, Yoshida M. Nonspecific integration of the HTLV provirus genome into adult T-cell leukaemia cells. *Nature* 1984; **309**: 640–642.
- 38 Neely SM. Adult T-cell leukemia/lymphoma. *West J Med* 1989; **150**: 557–561.
- 39 Okayama A, Tachibana N, Ishihara S, Nagatomo Y, Murai K, Okamoto K, Shima T, Sagawa K, Tsubouchi H, Stuver S, Mueller N. Increase expression of interleukin-2 receptor alpha on peripheral blood mononuclear cells in HTLV-I tax/rex mRNA-positive asymptomatic carriers. *J Acquir Immune Defic Syndr Hum Retrovirology* 1997; **15**: 70–75.
- 40 Yip MT, Chen ISY. Modes of transformation by the human T-cell leukemia viruses. *Mol Biol Med* 1990; **7**: 33–44.
- 41 Tajima K, The T- and B-cell malignancy Study Group. The fourth nationwide study of adult T-cell leukemia/lymphoma (ATL in Japan): estimates of risk of ATL and its geographical and clinical features. *Int J Cancer* 1990; **45**: 237–243.
- 42 Tokudome S, Tokunaga O, Shimamoto Y, Miyamoto Y, Sumida I, Kikuchi M, Takeshita M, Ikeda T, Fujiwara K, Yoshihara M, Yanagawa T, Nishizumi M. Incidence of adult T-cell leukemia/lymphoma among human T-lymphotropic virus type 1 carriers in Saga, Japan. *Cancer Res* 1989; **49**: 226–228.
- 43 Hinuma Y, Nagata K, Hanaoka M, Nakai M, Matsumoto K, Kuoshita KI, Shirakawa S, Miyoshi I. Antigen in an ATL cell line and detection of antibodies to the antigen in human sera. *Proc Natl Acad Sci USA* 1981; **78**: 6476–6480.
- 44 Okamoto T. Multi-step carcinogenesis model for adult T-cell leukemia. *Jpn J Cancer Res* 1989; **80**: 191–195.
- 45 Hunter T, Pines J. Cyclins and cancer II. Cyclin D and CDK inhibitors come of age. *Cell* 1994; **79**: 573–582.
- 46 Sherr CJ. G1 phase progression: cycling on cue. *Cell* 1994; **79**: 551–555.
- 47 Matsushime H, Roussel MF, Ashmun RA, Sherr CJ. Colony-stimulating factor 1 regulates novel cyclins during the G1 phase of the cell cycle. *Cell* 1991; **65**: 701–713.
- 48 Nurse P. Ordering S phase and M phase in the cell cycle. *Cell* 1994; **79**: 547–550.
- 49 Roberts JM, Koff A, Polyak K, Firpo R, Collins S, Ohtsubo M, Massague J. Cyclins, CDKs, and cyclin kinase inhibitors. *Cold Spring Harb Symp Quant Biol* 1994; **59**: 31–38.
- 50 Morgan DO. Principles of CDK regulation. *Nature* 1995; **374**: 131–134.
- 51 Cordon-Cardo C. Mutation of cell cycle regulators. Biological and clinical implications for human neoplasia. *Am J Pathol* 1995; **147**: 545–560.
- 52 Biggs JR, Kraft AS. Inhibitors of cyclin-dependent kinase and cancer. *J Mol Med* 1995; **73**: 509–514.
- 53 Yang R, Morosetti R, Koeffler HP. Characterization of a second human cyclin A that is highly expressed in testis and in several leukemic cell lines. *Cancer Res* 1997; **57**: 913–920.
- 54 Xiong Y, Zhang H, Beach D. D-type cyclins associates with multiple protein kinase and the DNA replication and repair factor PCNA. *Cell* 1992; **71**: 505–514.
- 55 Pines J. Cyclins and cyclin-dependent kinases: take your partners. *Trends Biochem Sci* 1993; **18**: 195–197.
- 56 Sherr CJ. Mammalian G1 cyclins. *Cell* 1993; **73**: 1059–1065.
- 57 Meyerson M, Harlow E. Identification of a G1 kinase activity for cdk6, a novel cyclin D partner. *Mol Cell Biol* 1994; **14**: 2077–2086.
- 58 Quelle DE, Ashmun RA, Shurtleff SA, Kto J, Bar-Sagi D, Roussel MF, Sherr CJ. Overexpression of mouse D-type cyclins accelerates G1 phase in rodent fibroblasts. *Genes Dev* 1996; **7**: 1559–1571.
- 59 Resnitzky D, Gossen M, Bujard H, Reed SI. Acceleration of the G1 to S phase transition by expression of cyclins D1 and E with an inducible system. *Mol Cell Biol* 1994; **14**: 1669–1679.
- 60 Matsushime H, Quelle DE, Shurtleff SA, Shibuya M, Sherr CJ, Kato JY. D-type cyclin-dependent kinase activity in mammalian cells. *Mol Cell Biol* 1994; **14**: 2066–2076.
- 61 Dulic V, Lees E, Reed SI. Association of human cyclin E with a periodic G1-S phase protein kinase. *Science* 1992; **257**: 1958–1961.
- 62 Girard F, Strausfeld U, Fernandez A, Lamb NJ. Cyclin A is required for the onset of DNA replication in mammalian fibroblast. *Cell* 1991; **67**: 1169–1179.
- 63 Dowdy SF, Hinds PW, Louis K, Reed SI, Weinberg RA. Physical interactions of the retinoblastoma protein with human cyclins. *Cell* 1993; **73**: 499–511.
- 64 Ewen ME, Sluss HK, Sherr CJ, Matsushime H, Kato J, Livingston DM. Functional interaction of the retinoblastoma protein with mammalian D-type cyclin. *Cell* 1993; **73**: 487–497.
- 65 Nevins JR. E2F: a link between the Rb tumor suppressor protein and viral oncoproteins. *Science* 1992; **258**: 424–429.
- 66 Hinds PW, Weinberg RA. Tumor suppressor genes. *Curr Opin Genet Dev* 1994; **4**: 135–141.
- 67 Dyson N, Howley PM, Munger K, Harlow E. The human papilloma virus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. *Science* 1989; **243**: 934–937.
- 68 Whyte P, Williamson NM, Harlow E. Cellular targets for transformation by the adenovirus E1A proteins. *Cell* 1989; **56**: 67–75.
- 69 Akagi T, Ono H, Shimotohno K. Expression of cell-cycle regulatory genes in HTLV-I infected T-cell lines: possible involvement of Tax1 in the altered expression of cyclin D2, p18^{INK4} and p21^{Waf1/Cip1/Sdi1}. *Oncogene* 1996; **12**: 1645–1652.
- 70 Suzuki T, Kitao S, Matsushime H, Yoshida M. HTLV-1 Tax protein interacts with cyclin-dependent kinase inhibitor p16^{INK4A} and counteracts its inhibitory activity towards CDK4. *EMBO J* 1996; **15**: 1607–1614.

- 71 Hannon GJ, Beach D. p15^{INK4B} is a potential effector of TGF- β -induced cell cycle arrest. *Nature* 1994; **371**: 257–261.
- 72 Serrano M, Hannon GJ, Beach D. A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. *Nature* 1993; **366**: 704–707.
- 73 Guan K-L, Jenkins CW, Li Y, Nichols MA, Wu X, O'Keefe CL, Matera AG, Xiong Y. Growth suppression by p18, a p16^{INK4/MTS1}- and p14^{INK4/MTS2}-related CDK6 inhibitor, correlates with wild-type pRb function. *Gen Dev* 1994; **8**: 2939–2952.
- 74 Hirai H, Roussel MF, Kato J, Ashmun RA, Sherr CJ. Novel INK4 proteins, p19 and p18, are specific inhibitors of cyclin D-dependent kinases CDK4 and CDK6. *Mol Cell Biol* 1995; **15**: 2672–2681.
- 75 Chan FK, Zhang J, Cheng L, Shapiro DN, Winoto A. Identification of human and mouse p19, a novel CDK4 and CDK6 inhibitor with homology to p16^{ink4}. *Mol Cell Biol* 1995; **15**: 2682–2688.
- 76 Yang R, Gombart AF, Serrano M, Koeffler HP. Mutational effects on the p16^{INK4a} tumor suppressor protein. *Cancer Res* 1995; **55**: 2503–2506.
- 77 Serrano M, Gornall-Lahoz E, DePinho RA, Beach D, Bar-Sagai D. Inhibition of Ras-induced proliferation and cellular transformation by p16^{INK4}. *Science* 1995; **267**: 249–252.
- 78 Koh J, Enders GH, Dynlacht BD, Harlow E. Tumor-derived p16 alleles encoding proteins defective in cell-cycle inhibition. *Nature* 1995; **375**: 506–510.
- 79 Lukas J, Parry D, Aagaard L, Mann DJ, Bartkova J, Strauss M, Peters G, Bartek J. Retinoblastoma-protein-dependent cell-cycle inhibition by the tumor suppressor p16. *Nature* 1995; **375**: 503–506.
- 80 Medema RH, Herrera RE, Lam F, Weingerg RA. Growth suppression by p16^{ink4} requires functional retinoblastoma protein. *Proc Natl Acad Sci USA* 1995; **92**: 6289–6293.
- 81 Gombart AF, Yang R, Campbell MJ, Berman JD, Koeffler HP. Inhibition of growth of human leukemia cell lines by retrovirally expressed wild-type p16^{INK4A}. *Leukemia* 1997; **11**: 1673–1680.
- 82 Kamb A, Gruis NA, Weaver-Feldhaus J, Liu Q, Harshman K, Tavtigian SV, Stockert E, Day RS3, Johnson BE, Skolnick MH. A cell cycle regulator potentially involved in genesis of many tumor types. *Science* 1994; **264**: 436–440.
- 83 Nobori T, Miura K, Wu DJ, Lois A, Takabayashi K, Carson DA. Deletion of the cyclin-dependent kinase-4 inhibitor gene in multiple human cancers. *Nature* 1994; **368**: 753–756.
- 84 Kamb A, Shattuck-Eicens D, Eeles R, Liu Q, Gruis N, Ding W, Hussey C. Analysis of the p16 gene (CDKN2) as a candidate for the chromosome 9p melanoma susceptibility locus. *Nat Genet* 1994; **8**: 23–26.
- 85 Okamoto A, Demetrick DJ, Splillare EA, Hagiwara K, Hussain SP, Bennett WP, Forrester K, Gerwin B, Serrano M, Beach D, Harris CC. Mutations and altered expression of p16^{INK4} in human cancer. *Proc Natl Acad Sci USA* 1994; **91**: 11045–11049.
- 86 van der Riet P, Nawroz H, Hruban H, Corio R, Tokino K, Koch W, Sidransky D. Frequent loss of chromosome 9p21–22 early in head and neck cancer progression. *Cancer Res* 1994; **54**: 1156–1158.
- 87 Takeuchi S, Bartram CR, Wada M, Reiter A, Hata Y, Seriu T, Lee E, Miller CW, Miyoshi I, Koeffler HP. Allelotyping analysis of childhood acute lymphoblastic leukemia. *Cancer Res* 1995; **55**: 5377–5382.
- 88 Sheaff RJ, Roberts JM. Tumor suppression. Lessons in p16 from phylum. *Falconium Curr Biol* 1995; **5**: 28–31.
- 89 Chaganti SR, Gaidano G, Loie DC, Dalla-Favera R, Chaganti RSK. Diffuse large cell lymphomas exhibit frequent deletions in 9p21–22 and 9q31–34 regions. *Genes Chromosom Cancer* 1995; **12**: 32–36.
- 90 Hata Y, Hirma T, Miller CW, Yamada Y, Tomonaga M, Koeffler HP. Homozygous deletions of the p15 (MTS2) and p16 (CDKN2/MTS1) genes in adult T-cell leukemia (ATL). *Blood* 1995; **85**: 2699–2704.
- 91 Gombart AF, Morosetti R, Miller CW, Said JW, Koeffler P. Deletions of the cyclin-dependent kinase inhibitor genes p16^{INK4A} and p15^{INK4B} in non-Hodgkin's lymphomas. *Blood* 1995; **86**: 1534–1539.
- 92 Takeuchi S, Bartram CR, Seriu T, Miller CW, Tobler A, Janssen JWG, Reiter A, Ludwig W, Zimmermann M, Schwaller J, Lee E, Miyoshi I, Koeffler HP. Analysis of a family of cyclin-dependent kinase inhibitors: p15/MTS2/INK4B, p16/MTS1/INK4A and p18 genes in acute lymphoblastic leukemia (ALL) of childhood. *Blood* 1995; **86**: 755–760.
- 93 Koduru PRK, Zariwala M, Soni M, Gong JZ, Xiong Y, Broome JD. Deletion of cyclin-dependent kinase 4 inhibitor genes p15 and p16 in non-Hodgkin's lymphoma. *Blood* 1995; **86**: 2900–2905.
- 94 Khatib ZA, Matsudhime H, Valentine M, Shapiro DN, Sherr CJ, Look AT. Coamplification of the CDK4 gene with MDM2 and GLI in human sarcomas. *Cancer Res* 1993; **55**: 5535–5541.
- 95 Otterson GA, Kratzke RA, Coxon A, Kim YW, Kaye FJ. Absence of p16^{INK4} protein is restricted to the subset of lung cancer lines that retains wildtype RB. *Oncogene* 1994; **9**: 3375–3378.
- 96 Hirma T, Koeffler HP. Role of the cyclin-dependent kinase inhibitors in the development of cancer. *Blood* 1995; **86**: 841–854.
- 97 Parry D, Bates S, Mann DJ, Peters G. Lack of cyclin D-Cdk complexes in Rb-negative cells correlates with high levels of p16^{INK4/MTS1} tumor suppressor gene product. *EMBO J* 1995; **14**: 503–511.
- 98 Miller CW, Aslo A, Campbell MJ, Kawamata N, Lampkin BC, Koeffler HP. Alterations of the p15, p16, and p18 genes in osteosarcoma. *Cancer Genet Cytogenet* 1996; **86**: 136–142.
- 99 Otsuki T, Jaffe ES, Wellmann A, Kumar S, Condron KS, Raffeld M. Absence of p18 mutations or deletions in lymphoid malignancies. *Leukemia* 1996; **10**: 356–360.
- 100 Iolascon A, Faienza MF, Coppola B, Della Ragione F, Schettini F, Biondi A. Homozygous deletions of cyclin-dependent kinase inhibitor genes p16^{INK4A} and p18, in childhood T cell lineage acute lymphoblastic leukemias. *Leukemia* 1996; **10**: 255–260.
- 101 Tasaka T, Berenson J, Vesico R, Hirma T, Miller CW, Nagai M, Takahara J, Koeffler HP. Analysis of the p16^{INK4A}, p15^{INK4B} and p18^{INK4C} genes in multiple myeloma. *Br J Haematol* 1997; **96**: 98–102.
- 102 Okuda T, Hirai H, Valentine VA, Shurtleff SA, Kidd VJ, Lahti JM, Sherr CJ, Downing JR. Molecular cloning, expression pattern, and chromosomal localization of human CDKN2D/INK4d, an inhibitor of cyclin D-dependent kinases. *Genomics* 1995; **29**: 623–630.
- 103 Shiohara M, Spirin K, Said JW, Gombart AF, Nakamaki T, Takeuchi S, Hata Y, Morosetti R, Tasaka T, Seriu T, Bartram C, Miller CW, Tomonaga M, Koeffler HP. Alterations of the cyclin-dependent kinase inhibitor p19 (INK4D) is rare in hematopoietic malignancies. *Leukemia* 1996; **10**: 1897–1900.
- 104 Zariwala M, Xiong Y. Lack of mutation in the cyclin-dependent kinase inhibitor, p19^{INK4d}, in tumor-derived cell lines and primary tumors. *Oncogene* 1996; **13**: 2033–2038.
- 105 El-Deiry WS, Tokino T, Velculescu V, Levy DB, Parsons R, Trent JM, Lin D, Mercer WE, Kinzler KW, Vogelstein B. WAF1, a potential mediator of p53 tumor suppression. *Cell* 1993; **75**: 817–825.
- 106 Harper JW, Adami GR, Wei N, Keyomarsi K, Elledge S. The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. *Cell* 1993; **75**: 805–816.
- 107 Gu Y, Turck CW, Morgan DO. Inhibition of CDK2 activity *in vivo* by an associated 20K regulatory subunit. *Nature* 1993; **366**: 707–710.
- 108 Noda A, Ning Y, Venables SF, Pereira-Smith OM, Smith JR. Cloning of senescent cell-derived inhibitors of DNA synthesis using an expression screen. *Exp Cell Res* 1994; **211**: 90–98.
- 109 Polyak K, Lee MH, Erdjument-Bromage H, Koff A, Roberts JM, Massague J. Cloning of p27^{Kip1}, a cyclin-dependent kinase inhibitor and a potential mediator of extracellular antimitogenic signals. *Cell* 1994; **78**: 59–66.
- 110 Toyoshima H, Hunter T. p27, a novel inhibitor of G1 cyclin-cdk protein kinase activity, is related to p21. *Cell* 1994; **78**: 67–74.
- 111 Lee MH, Reynisdottir I, Massague J. Cloning of p57^{Kip2}, a cyclin-dependent kinase inhibitor with unique domain structure and tissue distribution. *Genes Dev* 1995; **9**: 639–649.
- 112 Matsuoka S, Edwards M, Bai C, Parker S, Zhang P, Baldini A, Harper JW, Elledge SJ. p57^{Kip2}, a structurally distinct member of the p21^{Cip1} cdk inhibitory family, is a candidate tumor suppressor gene. *Genes Dev* 1995; **9**: 650–662.
- 113 Zhang H, Xiong Y, Beach D. Proliferating cell nuclear antigen and p21 are components of multiple cell cycle kinase complexes. *Mol Biol Cell* 1993; **4**: 897–906.

- 114 Xiong H, Zhang H, Beach D. Subunit rearrangement of the cyclin-dependent kinase is associated with cellular transformation. *Genes Dev* 1993; **7**: 1572–1583.
- 115 Chen J, Jackson PK, Kirshner MW, Dutta A. Separate domains of p21 involved in the inhibition of Cdk kinase and PCNA. *Nature* 1995; **374**: 386–388.
- 116 Luo Y, Hurwitz J, Massague J. Cell-cycle inhibition by independent CDK and PCNA binding domains in p21^{Cip1}. *Nature* 1995; **375**: 159–161.
- 117 Michieli P, Chedid M, Lin D, Pierce JH, Mercer WE, Givol D. Induction of WAF1/CIP1 by a p53 independent pathway. *Cancer Res* 1994; **54**: 3391–3395.
- 118 Macleod KF, Sherry N, Hannon G, Beach D, Tokino T, Kinzler K, Vogelstein B, Jacks T. p53 dependent and independent expression of p21 during cell growth, differentiation, and DNA damage. *Genes Dev* 1995; **9**: 935–944.
- 119 Akashi M, Osawa Y, Koeffler HP, Hachiya M. p21^{WAF1} expression by an activator of protein kinase C is regulated mainly at the post-transcriptional level in cells lacking p53: important role of RNA stabilization. *Biochem J* 1999; **337**: 607–616.
- 120 Kato JY, Matsuoka M, Polyak K, Massague J, Sherr CJ. Cyclic AMP-induced G1 phase arrest mediated by an inhibitor (p27^{Kip1}) of cyclin-dependent kinase 4 activation. *Cell* 1994; **79**: 487–496.
- 121 Polyak K, Kato JY, Solomon M, Sherr CJ, Massague JM, Koff A. p27^{Kip1}, a cyclin-Cdk inhibitor, links transforming growth factor- β and contact inhibition to cell cycle arrest. *Genes Dev* 1994; **8**: 9–22.
- 122 Zhang H, Hannon G, Beach D. p21-containing cyclin kinases exist in both active and inactive states. *Genes Dev* 1994; **8**: 1750–1758.
- 123 LaBaer J, Garrett MD, Stevenson LF, Slingerland JM, Sandhu C, Chou HS, Fattaeh A, Harlow E. New functional activities for the p21 family of CDK inhibitors. *Genes Dev* 1997; **11**: 847–862.
- 124 Shiohara M, El-Deiry WS, Wada M, Nakamaki T, Takeuchi S, Yang R, Chen D-L, Koeffler HP. Absence of WAF1 mutations in a variety of human malignancies. *Blood* 1994; **84**: 3781–3784.
- 125 Kawamata N, Morosetti R, Miller CW, Park D, Spirin KS, Nakamaki T, Takeuchi S, Hatta Y, Simpson J, Wilczynski S, Lee YY, Bartram CR, Koeffler HP. Molecular analysis of the cyclin-dependent kinase inhibitor gene p27^{Kip1} in human malignancies. *Cancer Res* 1995; **55**: 2266–2269.
- 126 Thompson JS, Reese KJ, DeBaun MR, Perlman EJ, Feinberg AP. Reduced expression of the cyclin-dependent kinase inhibitor gene p57^{KIP2} in Wilms' tumor. *Cancer Res* 1996; **56**: 5723–5727.
- 127 Orlow I, Iavarone A, Crider-Miller SJ, Bonilla F, Latres E, Lee MH, Gerald WL, Massague J, Weissman BE, Cordon-Cardo C. Cyclin-dependent kinase inhibitor p57^{KIP2} in soft tissue sarcomas and Wilms' tumors. *Cancer Res* 1996; **56**: 1219–1221.
- 128 Kastan MB, Zhan Q, El-Deiry WS, Carrier F, Jacks T, Walsh WV, Plunkett BS, Vogelstein B, Fornace AJ. A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia. *Cell* 1992; **71**: 587–597.
- 129 Carrier F, Smith ML, Bae I, Kilpatrick KE, Lansing TJ, Chen CY, Engelstein M, Friend SH, Henner WD, Gilmer TM, Kastan MB, Fornace AJ. Characterization of human Gadd45, a p53-regulated protein. *J Biol Chem* 1994; **269**: 32672–32677.
- 130 Masuda H, Miller CW, Koeffler HP, Battifora H, Cline MJ. Rearrangement of the p53 gene in human osteogenic sarcoma. *Proc Natl Acad Sci USA* 1987; **84**: 7716–7719.
- 131 Miller CW, Aslo K, Tsay C, Slamon D, Ishizaki K, Toguchida J, Yamamuro T, Lampkin B, Koeffler HP. Frequency and structure of p53 rearrangements in human osteosarcoma. *Cancer Res* 1990; **50**: 7950–7954.
- 132 Fagin JA, Matsuo K, Karmakar A, Chen D-L, Tang SH, Koeffler HP. High prevalence of mutations of the p53 gene in poorly differentiated human thyroid carcinomas. *J Clin Invest* 1993; **91**: 179–184.
- 133 Imamura J, Bartram CR, Berthold F, Harms D, Nakamura H, Koeffler HP. Mutation of the p53 gene in neuroblastoma and its relationship with N-myc amplification. *Cancer Res* 1993; **53**: 4053–4058.
- 134 Wada M, Bartram CR, Nakamura H, Hachiya M, Chen D, Borstein J, Miller CW, Ludwig L, Hansen-Hagge TE, Ludwig W-D, Reiter A, Mizoguchi H, Koeffler HP. Analysis of p53 mutations in a large series of lymphoid hematologic malignancies of childhood. *Blood* 1993; **82**: 3163–3169.
- 135 Imamura J, Miyoshi I, Koeffler HP. p53 in hematologic malignancies. *Blood* 1994; **84**: 2412–2421.
- 136 Michalovitz D, Halevy O, Oren M. p53 mutations: gains or loss? *J Cell Biochem* 1991; **45**: 22–29.
- 137 de Vos S, Miller CW, Takeuchi S, Gombart AF, Cho SK, Koeffler H. Alterations of CDKN2 (p16) in non-small-cell lung cancer. *Genes Chromosomes Cancer* 1995; **14**: 164–170.
- 138 Miller CW, Koeffler CW. Cyclin-dependent kinase inhibitors in human neoplasms. *Leukemia* 1997; **11** (Suppl. 3): 370–371.
- 139 Ogawa S, Hirano N, Sato N, Takahashi T, Hangaishi A, Tanaka K, Kurokawa M, Tanaka T, Mitani K, Yazaki Y, Hirai H. Homozygous loss of the cyclin-dependent kinase 4-inhibitor (p16) gene in human leukemia. *Blood* 1994; **84**: 2431–2435.
- 140 Hebert J, Cayuela JM, Berkeley J, Sigaux F. Candidate tumor-suppressor genes MTS1 (p16^{INK4A}) and MTS2 (p15^{INK4B}) display frequent homozygous deletions in primary cells from T- but not from B-cell lineage acute lymphoblastic leukemias. *Blood* 1994; **84**: 4038–4044.
- 141 Quesnel B, Preudhomme C, Philippe N, Vanrumbeke M, Dervite I, Lai JL, Bateurs F, Wattel E, Fenaux P. p16 gene homozygous deletions in acute lymphoblastic leukemia. *Blood* 1995; **85**: 657–663.
- 142 Hider MA, Cao XB, Manshouri T, Chan LL, Glassman A, Kantarjian HM, Keating MJ, Beran MS, Albitar M. p16^{INK4A} and p15^{INK4B} gene deletions in primary leukemias. *Blood* 1995; **86**: 311–315.
- 143 Otsuki T, Clark HM, Wellmann A, Jaffe ES, Raffeld M. Involvement of CDK2 (p16^{INK4A/MTS1}) and p15^{INK4B/MTS2} in human leukemias and lymphomas. *Cancer Res* 1995; **55**: 1436–1440.
- 144 Cayuela JM, Madani A, Sanhes L, Stern MH, Sigaux F. Multiple tumor suppressor gene 1 inactivation is the most frequent genetic alteration in T-cell acute lymphoblastic leukemia. *Blood* 1996; **87**: 2180–2186.
- 145 Ohnishi H, Kawamura M, Ida K, Sheng XM, Hanada R, Nobori T, Yamamori S, Hayashi Y. Homozygous deletions of p16/MTS1 gene are frequent but mutations are infrequent in childhood T-cell acute lymphoblastic leukemia. *Blood* 1995; **86**: 1269–1275.
- 146 Yamada Y, Hatta Y, Murata K, Sugawara K, Kamihira S, Ikeda S, Mine M, Maeda T, Hirakata Y, Kamihira S, Tsukasaki K, Ogawa S, Hirai H, Koeffler HP, Tomonaga M. Deletions of p15 and/or p16 genes as a poor-prognosis factor in adult T-cell leukemia. *J Clin Oncol* 1997; **15**: 1778–1785.
- 147 Fujiwara H, Arima N, Hashimoto-Tamaoki T, Matusita K, Ohtsubo H, Arimura K, Hidaka S, Tei C. Alteration of p16 (CDKN2) gene is associated with interleukin-2-induced tumor cell growth in adult T-cell leukemia. *Exp Hematol* 1999; **27**: 1004–1009.
- 148 Hangaishi A, Ogawa S, Imamura N, Miyawaki S, Miura Y, Uike N, Shimazaki C, Emi N, Takeyama K, Hirose S, Kamada N, Kobayashi Y, Takemoto Y, Kitani T, Toyama K, Ohtake S, Yazaki Y, Ueda R, Hirai H. Inactivation of multiple tumor-suppressor genes involved in negative regulation of the cell cycle, MTS1/p16^{INK4A/CDKN2}, MTS2/p15^{INK4B}, p53, and Rb genes in primary lymphoid malignancies. *Blood* 1996; **87**: 4949–4958.
- 149 Uchida T, Kinoshita T, Watanabe T, Nagai H, Murate T, Saito H, Hotta T. The CDKN2 gene alterations in various types of adult T-cell leukemia. *Br J Haematol* 1996; **94**: 665–670.
- 150 Cayuela JM, Herbert J, Sigaux F. Homozygous MTS1 (p16^{INK4A}) deletion in primary tumor cells of 163 leukemic patients. *Blood* 1995; **85**: 854.
- 151 Uchida T, Watanabe T, Kinoshita T, Murate T, Saito H, Hotta T. Mutational analysis of the CDKN2 (MTS1/p16^{INK4A}) gene in primary B-cell lymphoma. *Blood* 1995; **86**: 2724–2731.
- 152 Serra A, Gottardi E, Ragione FD, Saggio G, Iolasco A. Involvement of the cyclin-dependent kinase-4 inhibitor (CDKN2) gene in the pathogenesis of lymphoid blast crisis of chronic myelogenous leukaemia. *Br J Haematol* 1995; **91**: 625–629.
- 153 Sill H, Goldman JM, Cross NCP. Homozygous deletions of the p16 tumor-suppressor gene are associated with lymphoid transformation of chronic myeloid leukemia. *Blood* 1995; **85**: 2013–2016.
- 154 Stranks G, Height SE, Mitchell P, Jadayel D, Yuille MAL, DeLord C, Clutterbuck RD, Treleaven JG, Powels RL, Nacheva E, Oscier DG, Karpas A, Lenoir GM, Smith SD, Millar JL, Catovsky D, Dyer

- MJS. Deletions and rearrangements of CDKN2 in lymphoid malignancy. *Blood* 1995; **85**: 893–901.
- 155 Siebert R, Willers CP, Opalka B. Role of the cyclin-dependent kinase 4 and 6 inhibitor gene family p15, p16, p18 and p19 in leukemia and lymphoma. *Leuk Lymphoma* 1996; **23**: 505–520.
- 156 Drexler HG. Review of alterations of the cyclin-dependent kinase inhibitor INK4 family genes p15, p16, p18 and p19 in human leukemia-lymphoma cells. *Leukemia* 1998; **12**: 845–859.
- 157 Merlo A, Herman JG, Mao L, Lee DJ, Gabrielson E, Burger PC, Baylin SB, Sidransky D. 5'CpG island methylation is associated with transcriptional silencing of the tumour suppressor p16/CDKN2/MTS1 in human cancers. *Nat Med* 1995; **1**: 686–692.
- 158 Herman JG, Jen J, Merlo A, Baylin SB. Hypermethylation-associated inactivation indicates a tumor suppressor role for p15^{INK4B1}. *Cancer Res* 1996; **56**: 722–727.
- 159 Jones PA. DNA methylation errors and cancer. *Cancer Res* 1996; **56**: 2463–2467.
- 160 Herman JH, Civin CI, Issa JJ, Collector MI, Sharkis SJ, Baylin SB. Distinct patterns of inactivation of p15^{INK4B} and p16^{INK4A} characterize the major types of hematological malignancies. *Cancer Res* 1997; **57**: 837–841.
- 161 Batova A, Diccianni MB, Yu JC, Nobori T, Link MP, Pullen J, Yu AL. Frequent and selective methylation of p15 and deletion of both p15 and p16 in T-cell acute lymphoblastic leukemia. *Cancer Res* 1997; **57**: 832–836.
- 162 Tasaka T, Asou H, Munker R, Said JW, Berenson J, Nagai M, Takahara J, Koeffler HP. Methylation of the p16^{INK4A} gene in multiple myeloma. *Br J Haematol* 1998; **101**: 558–564.
- 163 Moller MB, Ino Y, Gerdes AM, Skjodt K, Louis DN, Pedersen NT. Aberrations of the p53 pathway components p53, MDM2 and CDKN2A appear independent in diffuse large B cell lymphoma. *Leukemia* 1999; **13**: 453–459.
- 164 Cameron EE, Baylin BS, Herman JG. p15^{INK4B} CpG island methylation in primary acute leukemias is heterogeneous and suggests density as a critical factor for transcriptional silencing. *Blood* 1999; **94**: 2445–2451.
- 165 Nosaka K, Maeda M, Tamiya S, Sakai T, Mitsuya H, Matsuoka M. Increasing methylation of the CDKN2 gene is associated with the progression of adult T-cell leukemia. *Cancer Res* 2000; **60**: 1043–1048.
- 166 Kawamata N, Miller CW, Koeffler HP. Molecular analysis of a family of cyclin-dependent kinase inhibitor genes (p15/MTS2/INK4b and p18/INK4c) in non-small cell lung cancers. *Mol Carcinog* 1995; **14**: 263–268.
- 167 Hata Y, Spirin K, Tasaka T, Morosetti R, Said JW, Yamada Y, Tomonaga M, Koeffler HP. Analysis of p18^{INK4C} in adult T-cell leukemia (ATL) and non-Hodgkin's lymphoma. *Br J Haematol* 1997; **99**: 665–667.
- 168 Suzuki T, Narita T, Uchida-Toita M, Yoshida M. Down-regulation of the INK4 family of cyclin-dependent kinase inhibitors by tax protein of HTLV-I through two distinct mechanisms. *Virology* 1999; **259**: 384–391.
- 169 Schwaller J, Pabst T, Koeffler HP, Niklaus G, Loetscher P, Fey MF, Tobler A. Expression and regulation of G1 cell-cycle inhibitors (p16^{INK4A}, p15^{INK4B}, p18^{INK4C}, p19^{INK4D}) in human acute myeloid leukemia and normal myeloid cells. *Leukemia* 1997; **11**: 54–63.
- 170 Zhao LJ, Giam CZ. Human T-cell lymphotropic virus type I (HTLV-I) transcriptional activator, Tax, enhances CREB binding to HTLV-I 21-base-pair repeats by protein-protein interaction. *Proc Natl Acad Sci USA* 1992; **89**: 7070–7074.
- 171 Fujii M, Tsuchiya H, Chuhjo T, Akizawa T, Seiki M. Interaction of HTLV-1 Tax with p67SRF causes the aberrant induction of cellular immediate early genes through CARG boxes. *Genes Dev* 1992; **6**: 2066–2076.
- 172 Suzuki T, Fujisawa J, Toita M, Yoshida M. The trans-activator tax of human T-cell leukemia virus type1 (HTLV-1) interacts with cAMP-responsive element (CRE) binding and CRE modulator proteins that bind to the 21-base-pair enhancer of HTLV-1. *Proc Natl Acad Sci USA* 1993; **90**: 610–614.
- 173 Suzuki T, Hirai H, Fujisawa J, Fujita T, Yoshida M. A trans-activator Tax of human T-cell leukemia virus type 1 binds to NF-kB p50 and serum response factor (SRF) and associates with enhancer DNAs of the NF-kB site and CARG box. *Oncogene* 1993; **8**: 2391–2397.
- 174 Suzuki T, Hirai H, Murakami T, Yoshida M. Tax protein of HTLV-1 destabilizes the complexes of NF-kB and Ikb-a and induces nuclear translocation of NF-kB for transcriptional activation. *Oncogene* 1995; **10**: 1199–1207.
- 175 Beg AA, Baldwin ASJ. The Ikb proteins: multifunctional regulators of Rel/NF-kB transcription factors. *Genes Dev* 1993; **7**: 2064–2070.
- 176 Hirai H, Suzuki T, Fujisawa J, Inoue J, Yoshida M. Tax protein of human T-cell leukemia virus type I binds to the ankyrin motifs of inhibitory factor kappa B and induces nuclear translocational activation. *Proc Natl Acad Sci USA* 1994; **91**: 3584–3588.
- 177 Höllsberg P, Ausubel LJ, Halfer DA. Human T cell lymphotropic virus type I-induced T cell activation. Resistance to TGF-β 1-induced suppression. *J Immunol* 1994; **153**: 566–573.
- 178 Murakami T, Hirai H, Suzuki T, Fujisawa J, Yoshida M. HTLV-I Tax enhances NF-kB2 expression and binds to the products p52 and p100, but does not suppress the inhibitory function of p100. *Virology* 1995; **206**: 1066–1074.
- 179 Uittenbogaard MN, Armstrong AP, Chiamarello A, Nyborg JN. Human T-cell leukemia virus type I Tax protein represses gene expression through the basic helix-loop-helix family of transcription factors. *J Biol Chem* 1994; **269**: 22466–22469.
- 180 Lemasson I, Robert-Hebmann V, Hamaia S, Duc Dodon M, Gazzolo L, Devaux C. Transrepression of lck gene expression by human T-cell leukemia virus type 1-encoded p40tax. *J Virol* 1997; **71**: 1975–1983.
- 181 Brauweiler A, Garrus JE, Reed JC, Nyborg JK. Repression of bax gene expression by the HTLV-I Tax protein: Implications for suppression of apoptosis in virally infected cells. *Virology* 1997; **231**: 135–140.
- 182 Xiong Y, Hannon GJ, Zhang H, Casso D, Kobayashi R, Beach D. p21 is a universal inhibitor of cyclin kinase. *Nature* 1993; **366**: 701–704.
- 183 Yang Z-Y, Perkins ND, Ohno T, Nabel EG, Nabel GJ. The p21 cyclin-dependent kinase inhibitor suppresses tumorigenicity *in vivo*. *Nat Med* 1995; **1**: 1052–1056.
- 184 Chen YQ, Cipriano SC, Arenkiel JM, Miller FR. Tumor suppression by p21^{WAF1}. *Cancer Res* 1995; **55**: 4536–4539.
- 185 Gartenhaus RB, Wang P, Hoffmann P. Induction of the WAF1/CIP1 protein and apoptosis in human T-cell leukemia virus type I-transformed lymphocytes after treatment with Adriamycin by using a p53-independent pathway. *Proc Natl Acad Sci USA* 1996; **93**: 265–268.
- 186 Tenan M, Carrara F, DiDonato S, Finocchiaro G. Absence of mutations and identification of two polymorphisms in the SSCP and sequence analysis of p21^{CK1} gene in malignant gliomas. *Int J Cancer* 1995; **62**: 115–117.
- 187 Bhatia K, Fan S, Spangler G, Weintraub M, O'Connor PM, Judde JG, Magrath I. A mutant p21 cyclin-dependent kinase inhibitor isolated from a Burkitt's lymphoma. *Cancer Res* 1995; **55**: 1431–1435.
- 188 Shiohara M, Koike K, Komiyama A, Koeffler HP. p21^{WAF1} mutations and human malignancies. *Leuk Lymphoma* 1997; **26**: 35–41.
- 189 Cereseto A, Diella F, Mulloy JC, Cara A, Michieli P, Grassmann R, Franchini G, Klotman ME. p53 functional impairment and high p21^{Waf1/Cip1} expression in human T-cell lymphotropic/leukemia virus type I-transformed T cells. *Blood* 1996; **88**: 1551–1560.
- 190 Jung JM, Bruner JM, Ruan S, Langford LA, Kyritsis AP, Kobayashi T, Levin VA, Zhang W. Increased levels of p21^{Waf1/Cip1} in human brain tumors. *Oncogene* 1995; **11**: 2021–2028.
- 191 Zhang W, Kornblau SM, Kobayashi T, Gambel A, Claxton D, Deisseroth AB. High level of constitutive WAF1/CIP1 protein are associated with chemoresistance in acute myelogenous leukemia. *Clin Cancer Res* 1995; **1**: 1051–1057.
- 192 Steinman RA, Hoffman B, Iro A, Guillof C, Liebermann DA, El-Houseini ME. Induction of p21 (WAF-1/CIP1) during differentiation. *Oncogene* 1994; **9**: 3389–3396.
- 193 Lin D, Fiscella M, O'Conner PM, Jackman J, Chen M, Luo LL, Sala A, Travali S, Appella E, Mercer WE. Constitutive expression of B-myb can bypass p53-induced Waf1/Cip1-mediated G1 arrest. *Proc Natl Acad Sci USA* 1994; **91**: 10079–10083.

- 194 Wolfel T, Hunter M, Schneider J, Serrano M, Wolfel C, Klehmann Hieb E, De Plaen E, Hankeln T, Meyer zum Buschenfelde K, Beach D. A p16INK4a-insensitive CDK4 mutant targeted by cytolytic T lymphocytes in a human melanoma. *Science* 1995; **269**: 1281–1284.
- 195 Zuo L, Weger J, Yang Q, Goldstein AM, Tucker MA, Walker GJ, Hayward N, Dracopoli NC. Germline mutation in the p16INK4a binding domain of CDK4 in familial melanoma. *Nat Genet* 1996; **12**: 97–99.
- 196 Di Leonardo A, Linke SP, Clarkin K, Wahl GM. DNA damage triggers a prolonged p53-dependent G1 arrest and long-term induction of Cip1 in normal human fibroblast. *Genes Dev* 1994; **8**: 2540–2551.
- 197 Dulic V, Kaufmann WK, Wilson SJ, Tlsty TD, Lees E, Harper W, Elledge SJ, Reed SI. p53-dependent inhibition of cyclin-dependent kinase activities in human fibroblasts during radiation-induced G1 arrest. *Cell* 1994; **76**: 1013–1023.
- 198 Parker SF, Perkins ND, Gitlin SD, Nabel GJ. A cooperative interaction of human T-cell leukemia virus type 1 tax with the p21 cyclin-dependent kinase inhibitor activates the human immunodeficiency virus type 1 enhancer. *J Virol* 1996; **70**: 5731–5734.
- 199 Yoshida M. Expression of the HTLV-I genome and its association with a unique T-cell malignancy. *Biochem Biophys Acta* 1987; **907**: 145–161.
- 200 Mansukhani M, Osborne B, Zhong J, Matsushima A. The pattern of p53 and p21^{WAF1/CIP1} immunoreactivity in non-Hodgkin's lymphomas predicts p53 gene status. *Diagn Mol Pathol* 1997; **6**: 222–228.
- 201 Loda M, Cuker B, Tam SW, Lavin P, Firentino M, Draetta GF, Jessup JM, Pageno M. Increased proteasome-dependent degradation of the cyclin-dependent kinase inhibitor p27 in aggressive colorectal carcinomas. *Nat Med* 1997; **3**: 231–234.
- 202 Morosetti R, Kawamata N, Gombert AF, Miller CW, Hatta Y, Hirama T, Said JW, Tomonaga M, Koeffler HP. Alterations of the p27^{KIP1} gene in non-Hodgkin's lymphoma and adult T-cell leukemia/lymphoma. *Blood* 1995; **86**: 1924–1930.
- 203 Iolascon A, Della-Ragione F, Giordani L, Serra A, Saglio G, Faienza MF. Expression of cell cycle regulatory gene in chronic myelogenous leukemia. *Haematologica* 1998; **83**: 771–777.
- 204 Marx J. How p53 suppresses cell growth. *Science* 1993; **262**: 1644–1645.
- 205 El-Deiry WS, Harper SW, O'Connor PM, Velculescu VE, Canman CE, Jackman J, Pietenpol JA, Burrell M, Hill DE, Wang Y, Wiman KG, Mercer WE, Kastan MB, Kohn KW, Elledge SJ, Kinzler KW, Tokino T, Vogelstein B. WAF1/CIP1 is induced in p53-mediated G1 arrest and apoptosis. *Cancer Res* 1994; **54**: 1169–1174.
- 206 Marx J. New link found between p53 and DNA repair. *Science* 1994; **266**: 1321–1322.
- 207 Greenblatt MS, Bennett WP, Hollstein M, Harris CC. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. *Cancer Res* 1994; **54**: 4855–4878.
- 208 Ichikawa A, Kinoshita T, Watanabe T, Kato H, Nagai H, Tsushita K, Saito H, Hotta T. Mutations of the p53 gene as a prognostic factor in aggressive B-cell lymphoma. *N Engl J Med* 1997; **337**: 529–534.
- 209 Reich NC, Oren M, Levine AJ. Two distinct mechanisms regulate the levels of a cellular tumor antigen, p53. *Mol Cell Biol* 1983; **3**: 2143–2150.
- 210 Halevy O, Hall A, Oren M. Stabilization of the p53 transformation-related protein in mouse fibrosarcoma cell lines: effects of protein sequence and intracellular environment. *Mol Cell Biol* 1989; **9**: 3385–3392.
- 211 Nagai H, Kinoshita T, Imamura J, Murakami Y, Hayashi K, Mukai K, Ikeda S, Tobinai K, Saito H, Shimoyama M, Shimotohno K. Genetic alteration of p53 in some patients with adult T-cell leukemia. *Jpn J Cancer Res* 1991; **82**: 1421–1427.
- 212 Sakashita A, Hattori T, Miller CW, Suzushima H, Asou N, Takatsuki K, Koeffler HP. Mutation of p53 gene in adult T-cell leukemia. *Blood* 1992; **79**: 477–480.
- 213 Sugito S, Yamato K, Sameshima Y, Yokota J, Yano S, Miyoshi I. Adult T-cell leukemia: structures and expression of the p53 gene. *Int J Cancer* 1991; **49**: 880–885.
- 214 Yamato K, Oka T, Hiroi M, Iwahara Y, Sugito S, Tsuchida N, Miyoshi I. Aberrant expression of the p53 tumor suppressor gene in adult T-cell leukemia and HTLV-I-infected cells. *Jpn J Cancer Res* 1993; **84**: 4–8.
- 215 Baker SJ, Preisinger AC, Jessup JM, Paraskeva C, Markowitz S, Willson JKV, Hamilton S, Vogelstein B. p53 gene mutations occur in combination with 17p allelic deletions as late events in colorectal tumorigenesis. *Cancer Res* 1990; **50**: 7717–7722.
- 216 Ichikawa A, Hotta T, Takagi N, Tsushita K, Kinoshita T, Nagai H, Murakami Y, Hayashi K, Saito H. Mutations of p53 gene and their relation to disease progression in B-cell lymphoma. *Blood* 1992; **79**: 2701–2707.
- 217 Lübert M, Miller CW, Kahan J, Koeffler HP. Expression, methylation and chromatin structure of the p53 gene in untransformed and human T-cell leukemia virus type I-transformed human T-lymphocytes. *Oncogene* 1989; **4**: 643–651.
- 218 Rideout WM 3rd, Coetzee GA, Olumi AF, Jones PA. 5-methylcytosine as an endogenous mutagen in human LDL receptor and p53 genes. *Science* 1990; **249**: 1288–1290.
- 219 Reid RL, Lindholm PF, Mireskandari A, Dittmer J, Brady JN. Stabilization of wild-type p53 in human T-lymphocytes transformed by HTLV-I. *Oncogene* 1993; **8**: 3029–3036.
- 220 Gartenhaus RB, Wang P. Functional inactivation of wild-type p53 protein correlates with loss of IL-2 dependence in HTLV-I transformed human T-lymphocytes. *Leukemia* 1995; **9**: 2082–2086.
- 221 Levine AJ, Momand J, Finlay CA. The p53 tumor suppressor gene. *Nature* 1991; **351**: 453–456.
- 222 Lowe SW, Schmitt EM, Smith SW, Osborne BA, Jacks T. p53 is required for radiation-induced apoptosis in mouse thymocytes. *Nature* 1993; **362**: 847–849.
- 223 Clarke AR, Purdie CA, Harrison DJ, Morris RG, Bird CC, Hooper ML, Wyllie AH. Thymocyte apoptosis induced by p53-dependent and independent pathways. *Nature* 1993; **362**: 849–852.
- 224 Akagi T, Ono H, Tsuchida N, Shimotohno K. Aberrant expression and function of p53 in T-cells immortalized by HTLV-I Tax1. *FEBS Lett* 1997; **406**: 263–266.
- 225 Chang F, Syrjanen S, Kurvinen K, Syrjanen K. The p53 tumor suppressor gene as a common cellular target in human carcinogenesis. *Am J Gastroenterol* 1993; **88**: 174–186.
- 226 Berneman Z, Gartenhaus RB, Reitz MS Jr, Blattner WA, Manns A, Hauchard B, Ikehara O, Gallo RC, Kotman ME. Expression of alternatively spliced human T-lymphotropic virus type I pX mRNA in infected cell lines and in primary uncultured cells from patients with adult T-cell leukemia/lymphoma and healthy carriers. *Proc Natl Acad Sci USA* 1992; **89**: 3005–3009.
- 227 Kaghad M, Bonnet H, Yang A, Creancier L, Bican J, Valent A, Minty A, Lelias J-M, Dumont X, Ferrara P, Mckeeon F, Caput D. Monoallelically expressed gene related to p53 at 1p36, a region frequently deleted in neuroblastoma and other human cancers. *Cell* 1997; **90**: 809–819.
- 228 Jost CA, Marin MC, Kaelin WJ Jr. p73 is a simian p53-related protein that can induce apoptosis. *Nature* 1997; **389**: 191–194.
- 229 Kawano S, Miller C, Bartram C, Matsuo Y, Asou H, Sakashita A, Said J, Tatsumi E, Koeffler H. Loss of p73 gene expression in leukemias/lymphomas due to hypermethylation. *Blood* 1999; **94**: 1113–1120.
- 230 Hatta Y, Yamada Y, Tomonaga M, Koeffler HP. Extensive analysis of the retinoblastoma gene in adult T-cell leukemia/lymphoma (ATL). *Leukemia* 1997; **11**: 984–989.
- 231 Greger V, Passarge E, Höpping W, Messmer E, Horsthemke B. Epigenetic changes may contribute to the formation and spontaneous regression of retinoblastoma. *Hum Genet* 1989; **83**: 155–158.
- 232 Sakai T, Toguchida J, Ohtani N, Yandell DW, Rapaport JM, Dryja TP. Allele-specific hypermethylation of the retinoblastoma tumor-suppressor gene. *Am J Hum Genet* 1991; **48**: 880–888.
- 233 Ohtani-Fujita N, Fujita T, Aoike A, Osifchin NE, Robbins PD, Sakai T. CpG methylation inactivates the promoter activity of the human retinoblastoma tumor-suppressor gene. *Oncogene* 1993; **8**: 1063–1067.
- 234 Scrabble HJ, Sapienza C, Cavenee WK. Genetic and epigenetic losses of heterozygosity in cancer predisposition and progression. *Adv Cancer Res* 1990; **54**: 25–62.
- 235 Benedict WF, Xu H-J, Hu S-X, Takahashi R. Role of the retinoblastoma gene in the initiation and progression of human cancer. *J Clin Invest* 1990; **85**: 988–993.

- 236 Kornblau SM, Chen A, del Giglio A, O'Brien S, Deisseroth AB. Retinoblastoma protein expression is frequently altered in chronic lymphocytic leukemia. *Cancer Res* 1994; **54**: 242–246.
- 237 Neubauer A, de Kant E, Rochlitz C, Laser J, Zanetta AM, Gallardo J, Oertel J, Herrmann R, Huhn D. Altered expression of the retinoblastoma susceptibility gene in chronic lymphocytic leukaemia. *Br J Haematol* 1993; **85**: 498–503.
- 238 Aagaard L, Lukas J, Bartkova J, Kjerulff AA, Strauss M, Bartek J. Aberrations of p16^{INK4} and retinoblastoma tumor-suppressor genes occur in distinct sub-sets of human cancer cell lines. *Int J Cancer* 1995; **61**: 115–120.
- 239 Shapiro GI, Edwards CD, Kobzik L, Godleski J, Richards W, Sugarbaker DJ, Rollins BJ. Reciprocal Rb inactivation and p16^{INK4} expression in primary lung cancers and cell lines. *Cancer Res* 1995; **55**: 505–509.
- 240 Schmidt EE, Ichimura K, Reifemberger G, Collins VP. CDKN2 (p16/MTS1) gene deletion or CDK4 amplification occurs in the majority of glioblastomas. *Cancer Res* 1994; **54**: 6321–6324.
- 241 Tam SW, Shay JW, Pagano M. Differential expression and cell cycle regulation of the cyclin-dependent kinase 4 inhibitor p16^{INK4}. *Cancer Res* 1994; **54**: 5816–5820.
- 242 Okamoto A, Hussain SP, Hagiwara K, Spillare EA, Rusin MR, Demetrick DJ, Serrano M, Hannon GJ, Shiseki M, Zariwala M, Xiong Y, Beach DH, Yokota J, Harris C. Mutation in the p16^{INK4}/MTS1/CDKN2, p15^{INK4B}/MTS2, and p18 genes in primary and metastatic lung cancer. *Cancer Res* 1995; **55**: 1448–1451.
- 243 Yeager T, Stadler W, Belair C, Puthenveetil J, Olopade O, Reznikoff C. Increased p16 levels correlate with pRb alterations in human urothelial cells. *Cancer Res* 1995; **55**: 493–497.
- 244 Williams BO, Remington L, Albert DM, Mukai S, Bronson RT, Jacks T. Cooperative tumorigenic effects of germline mutations in Rb and p53. *Nat Genet* 1994; **7**: 480–484.
- 245 Parsons R, Guo-Mon L, Longley ML, Fang W, Papadopoulos N, Jen J, de la Chapelle A, Kinzer KW, Vogelstein B, Modrich P. Hypermutability and mismatch repair deficiency in RER+ tumor cells. *Cell* 1993; **75**: 1227–1236.
- 246 Strand M, Prolla TA, Liskay RM, Petes TD. Destabilization of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair. *Nature* 1993; **365**: 274–276.
- 247 Bronner CE, Baker SM, Morrison PT, Warren G, Smith LG, Lescoe MK, Kane M, Earabino C, Lipford J, Lindlbom A, Tannergard P, Bollag RJ, Godwin AR, Ward DC, Nordenskjold M, Fishel R, Kolodner R, Liskay RM. Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer. *Nature* 1994; **368**: 258–261.
- 248 Papadopoulos N, Nicolaides NC, Wei Y-F, Ruben SM, Carter KC, Rosen CA, Haseltine WA, Fleischmann RD, Fraser CM, Adams MD, Venter JC, Hamilton SR, Peterson GM, Watson P, Lynch HT, Peltomäki P, Meklin J-P, de la Chapelle A, Kinzler KW, Vogelstein B. Mutation of a mutL homolog in hereditary colon cancer. *Science* 1994; **263**: 1625–1629.
- 249 Leach FS, Nicolaides NC, Papadopoulos N, Liu B, Jen J, Parsons R. Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. *Cell* 1993; **75**: 1215–1225.
- 250 Fishel R, Lescoe MK, Rao MRS, Copeland NG, Jenkins NA, Garber J, Kaned M, Kolodner R. The human mutator gene homologue MSH2 and its association with hereditary nonpolyposis colon cancer. *Cell* 1993; **75**: 1027–1038.
- 251 Horii A, Han H, Sasaki S, Shimada M, Nakamura Y. Cloning, characterization and chromosomal assignment of the human genes homologous to yeast PMS1, a member of mismatch repair genes. *Biochem Biophys Res Commun* 1994; **204**: 1257–1264.
- 252 Nicolaides NC, Papadopoulos N, Liu B, Wei YF, Carter KC, Ruben SM, Rosen CA, Haseltine WA, Fleischmann RD, Fraser CM, Adams MD, Venter JC, Dunlop MG, Hamilton SR, Petersen GM, de la Chapelle A, Vogelstein B, Kinzler KW. Mutations of two PMS homologues in hereditary nonpolyposis colon cancer. *Nature* 1994; **371**: 75–80.
- 253 Papadopoulos N, Nicolaides NC, Liu B, Parsons R, Lengauer C, Palombo F, D'Arrigo A, Markowitz S, Willson JKV, Kinzler KW, Jiricny J, Vogelstein B. Mutations of GTBP in genetically unstable cells. *Science* 1995; **268**: 1915–1917.
- 254 Reitmair AH, Schmits R, Ewel A, Bapat B, Redston M, Mitri A, Waterhouse P, Mittrücker H-W, Wakeham A, Liu B, Thomason A, Griesser H, Gallinger S, Ballhausen WG, Fishel R, Mak TW. MSH2 deficient mice are viable and susceptible to lymphoid tumours. *Nat Genet* 1995; **11**: 64–70.
- 255 de Wind N, Dekker M, Berns A, Radman M, te Riele H. Inactivation of the mouse Msh2 gene results in mismatch repair deficiency, methylation tolerance, hyperrecombination, and predisposition to cancer. *Cell* 1995; **82**: 321–330.
- 256 Baker SM, Plug AW, Prolla TA, Bronner CE, Harris AC, Yao X, Christite DM, Monell C, Arnheim N, Bradley A, Ashley T, Liskay RM. Involvement of mouse Mlh1 in DNA mismatch repair and meiotic crossing over. *Nat Genet* 1996; **13**: 336–342.
- 257 Edelmann W, Cohen PE, Kane M, Lau K, Morrow B, Bennett S, Umar A, Kunkel T, Cattoretti G, Chaganti R, Pollard JW, Koldner RD, Kucherlapati R. Meiotic pachytene arrest in MLH1-deficient mice. *Cell* 1996; **85**: 1125–1134.
- 258 Wada C, Shinoya S, Fujino H, Tokuiro H, Akahoshi T, Uchida T, Ohtani H. Genomic instability of microsatellite repeats and its association with the evolution of chronic myelogenous leukemia. *Blood* 1994; **83**: 3449–3456.
- 259 Tasaka T, Lee S, Spira S, Takeuchi S, Nagai M, Takahara J, Koeffler HP. Microsatellite instability during the progression of acute myelocytic leukaemia. *Br J Haematol* 1997; **98**: 219–221.
- 260 Bedi CG, Westra WH, Farzadegan H, Pitha P, Sidransky D. Microsatellite instability in primary neoplasms from HIV+ patients. *Nat Med* 1995; **1**: 65–68.
- 261 Takeuchi S, Seriu T, Tasaka T, Koike M, Cho SK, Park S, Slater J, Mufti J, Hata Y, Miyoshi I, Bartram CR, Koeffler HP. Microsatellite instability and other molecular abnormalities in childhood acute lymphoblastic leukaemia. *Br J Haematol* 1997; **98**: 134–139.
- 262 Hata Y, Yamada Y, Tomonaga M, Miyoshi I, Said JW, Koeffler HP. Microsatellite instability in adult T-cell leukemia (ATL). *Br J Haematol* 1998; **101**: 341–344.
- 263 Young J, Leggett B, Gustafson C, Ward M, Searle J, Thomas L, Buttenshaw R, Chenevix Trench G. Genomic instability occurs in colorectal carcinomas but not in adenomas. *Hum Mutat* 1993; **2**: 351–354.
- 264 Lynch H, Smyrk T, Watson P, Lanspa S, Lynch J, Lynch P, Cavalieri R, Boland C. Genetics, natural history, tumor spectrum, and pathology of hereditary nonpolyposis colorectal cancer: an updated review. *Gastroenterology* 1993; **104**: 1535–1549.
- 265 Shibata D, Peinado MA, Ionov Y, Malkhosyan S, Perucho M. Genomic instability in repeated sequences is an early somatic event in colorectal tumorigenesis that persists after transformation. *Nat Genet* 1994; **6**: 273–281.
- 266 Aaltonen LA, Peltomäki P, Mecklin JP, Jarvinen H, Jass JR, Green JS, Lynch HT, Watson P, Tallqvist G, Juhola M, Sistonen P, Hamilton SR, Kinzler KW, Vogelstein B, de la Chapelle A. Replication errors in benign and malignant tumors from hereditary nonpolyposis colorectal cancer patients. *Cancer Res* 1994; **54**: 1645–1648.
- 267 Chong J-M, Fukuyama M, Hayashi Y, Takizawa T, Koike M, Konishi M, Kikuchi-Yanoshita R, Miyaki M. Microsatellite instability in the progression of gastric carcinoma. *Cancer Res* 1994; **54**: 4595–4597.
- 268 Jass JR, Stewart SM, Stewart J, Lane MR. Hereditary non-polyposis colorectal cancer-morphologies, genes and mutations. *Mutat Res* 1994; **310**: 125–133.
- 269 Baker SM, Bronner CE, Zhang L, Plug AW, Robatzek M, Warren G, Elliott EA, Yu J, Ashley T, Arnheim N, Flavell RA, Liskay RM. Male mice defective in the DNA mismatch repair gene PMS2 exhibit abnormal chromosome synapsis in meiosis. *Cell* 1995; **82**: 309–313.
- 270 Lynch HT, Krush AJ. Cancer family 'G' revisited: 1895–1970. *Cancer* 1971; **27**: 1505–1511.
- 271 Law IP, Herberman RB, Oldham RK, Bouzoukis J, Hanson SM, Rhode MC. Familial occurrence of colon and uterine carcinoma and of lymphoproliferative malignancies: clinical description. *Cancer* 1977; **39**: 1224–1278.
- 272 Law IP, Hollinshead AC, Whang Peng J, Dean JH, Oldham RK, Herberman RB, Rhode MC. Familial occurrence of colon and uterine carcinoma and of lymphoproliferative malignancies. II. Chromosomal and immunologic abnormalities. *Cancer* 1977; **39**: 1229–1236.
- 273 Love RR. Small bowel cancers, B-cell lymphatic leukemia, and

- six primary cancers with metastases and prolonged survival in the cancer family syndrome of Lynch. *Cancer* 1985; **55**: 499–502.
- 274 Aaltonen LA, Peltomäki P, Leach FS, Sistonen P, Pylkkanen L, Mecklin J-P, Jarvinen H, Powell SM, Jen J, Hamilton SR, Petersen GM, Kinzler KW, Vogelstein B, de la Chapelle A. Clues to the pathogenesis of familial colorectal cancer. *Science* 1993; **260**: 812–816.
- 275 Borresen A, Lothe RA, Meling GI, Lystad S, Morrison P, Lipford J, Kane MF, Rognum TO, Kolander RD. Somatic mutations in the hMSH2 gene in microsatellite unstable colorectal carcinomas. *Hum Mol Genet* 1995; **4**: 2065–2072.
- 276 Liu B, Nicolaides MC, Markowitz S, Willson JKV, Parsons RE, Jen J, Papadopoulos N, Peltomäki P, de la Chapelle A, Hamilton SR, Kinzler KW, Vogelstein B. Mismatch repair gene defects in sporadic colorectal cancers with microsatellite instability. *Nat Genet* 1995; **9**: 48–55.
- 277 Katabuchi H, van Rees B, Lambers AR, Ronnet BM, Blazes MS, Leach FS, Cho KR, Hedrick L. Mutations in DNA mismatch repair genes are not responsible for microsatellite instability in most sporadic endometrial carcinomas. *Cancer Res* 1995; **55**: 5556–5560.
- 278 Moslein G, Tester DJ, Lindor NM, Honchel R, Cunningham JM, French AJ, Halling KC, Schwab M, Goretzki P, Thibodeau SN. Microsatellite instability and mutation analysis of hMSH2 and hMLH1 in patients with sporadic familial and hereditary colorectal cancer. *Hum Mol Genet* 1996; **5**: 1245–1252.
- 279 Kane FM, Loda M, Gaida GM, Lipman J, Mishra R, Goldman H, Jessup JM, Koldner R. Methylation of the hMLH1 promoter correlates with lack of expression of hMLH1 in sporadic colon tumors and mismatch repair-defective human tumor cell lines. *Cancer Res* 1997; **57**: 808–811.
- 280 Hatta Y, Wada M, Takeuchi S, Tasaka T, Lee E, Lee YY, Kim BK, Bnag Y-J, Lee S, Yamada Y, Tomonaga M, Wilczynski SP, Said JW, Koeffler HP. Mutational analysis of the hMSH2 gene in a wide variety of tumors. *Int J Oncol* 1997; **11**: 465–469.
- 281 Robledo M, Martinez B, Arranz E, Trujillo MJ, Gonzalez Ageitos A, Rivas C, Benitez J. Genetic instability of microsatellites in hematological neoplasms. *Leukemia* 1995; **9**: 960–964.
- 282 Hangaishi A, Ogawa S, Mitani K, Hosoya N, Chiba S, Yazaki Y, Hirai H. Mutations and loss of expression of a mismatch repair gene, hMLH1, in leukemia and lymphoma cell lines. *Blood* 1997; **89**: 1740–1747.
- 283 Liu B, Parsons R, Papadopoulos N, Nicolaides MC, Lynch HT, Watson P, Jass J, Dunlop M, Wyllie A, Peltomäki P, de la Chapelle A, Hamilton SR, Vogelstein B, Kinzler KW. Analysis of mismatch repair genes in hereditary non-polyposis colorectal cancer patients. *Nat Med* 1996; **2**: 169–174.
- 284 Komatsu N, Takeuchi S, Ikezoe T, Tasaka T, Hatta Y, Machida H, Williamson IK, Bartram CR, Koeffler HP, Taguchi H. Mutations of the E2F4 gene in hematological malignancies having microsatellite instability. *Blood* 2000; **95**: 1509–1510.
- 285 Saito S, Saito H, Koi S, Sagae S, Kudo R, Saito J, Noda K, Nakamura Y. Fine-scale deletion mapping of the distal long arm of chromosome 6 in 70 human ovarian cancers. *Cancer Res* 1992; **52**: 5815–5817.
- 286 Theile M, Seitz S, Arnold W, Jandrig B, Frege R, Schlag PM, Haensch W, Winzer K-J, Barrett JC, Scherneck S. A defined chromosome 6q fragment (at D6S310) harbors a putative tumor suppressor gene for breast cancer. *Oncogene* 1996; **13**: 677–685.
- 287 Ray ME, Su YA, Meltzer PS, Trent J. Isolation and characterization of genes associated with chromosome-6 mediated tumor suppression in human malignant melanoma. *Oncogene* 1996; **12**: 2527–2533.
- 288 Menasce LP, Orphanos V, Santibanez-Koref M, Boyle JM, Harrison CJ. Common region of deletion on the long arm of chromosome 6 in non-Hodgkin's lymphoma and acute lymphoblastic leukemia. *Genes Chromosomes Cancer* 1994; **10**: 286–288.
- 289 Gerard B, Cave H, Guidal C, Dastugue N, Vilmer E, Grandchamp B. Deletion of a 6cM commonly deleted region in childhood acute lymphoblastic leukemia on the 6q chromosomal arm. *Leukemia* 1997; **11**: 228–232.
- 290 Takeuchi S, Koike M, Seriu T, Bartram CR, Schrappe M, Reiter A, Park S, Taub HE, Miyoshi I, Koeffler HP. Frequent loss of heterozygosity on the long arm of chromosome 6: identification of two distinct regions of deletion in childhood acute lymphoblastic leukemia. *Cancer Res* 1998; **58**: 2618–2623.
- 291 Gaidano G, Hauptschein RS, Parsa NZ, Offit K, Rao PH, Lenoir G, Knowles DM, Chaganti RSK, Dalla-Favera R. Deletions involving two distinct regions of 6q in B-cell non-Hodgkin lymphoma. *Blood* 1992; **80**: 1781–1787.
- 292 Johansson B, F. M, Mitelman F. Cytogenetic deletion maps of hematologic neoplasms: circumstantial evidence of tumor suppressor loci. *Genes Chromosomes Cancer* 1993; **8**: 205–218.
- 293 Offit K, Parsa NZ, Gaidano G, Filippa DA, Louie D, Pan D, Jhanwar SC, Dalla-Favera R, Chaganti RSK. 6q deletions define distinct clinico-pathologic subsets of non-Hodgkin's lymphoma. *Blood* 1993; **82**: 2157–2162.
- 294 Guan X-Y, Horsman D, Zhang HE, Parsa NZ, Meltzer PS, Trent JM. Localization by chromosome microdissection of a recurrent breakpoint region on chromosome 6 in human B-cell lymphoma. *Blood* 1996; **88**: 1418–1422.
- 295 Sadamori N. Cytogenetic implication in adult T-cell leukemia: a hypothesis of leukemogenesis. *Cancer Genet Cytogenet* 1991; **51**: 131–136.
- 296 Kamada N, Sakurai M, Miyamoto K, Sanada I, Sadamori N, Fukuhara S, Abe S, Shiraishi Y, Abe T, Kaneko Y, Shimoyama M. Chromosome abnormalities in adult T-cell leukemia/lymphoma: a karyotype review committee report. *Cancer Res* 1992; **52**: 1481–1493.
- 297 Hatta Y, Yamada Y, Tomonaga M, Said JW, Miyoshi I, Koeffler HP. Allelotyping analysis of adult T-cell leukemia (ATL). *Blood* 1998; **92**: 2113–2117.
- 298 Hatta Y, Yamada Y, Tomonaga M, Miyoshi I, Said JW, Koeffler HP. Detailed deletion mapping of the long arm of chromosome 6 in adult T-cell leukemia (ATL). *Blood* 1999; **93**: 613–616.
- 299 Hahn SA, Schutte M, Hoque ATMS, Moskaluk CA, da Costa LT, Rozenblum E, Weinstein CL, Fischer A, Yeo CJ, Hruban RH, Kern SE. DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1. *Science* 1996; **271**: 350–353.
- 300 Verbeek W, Spirin K, Hatta Y, Miller CW, Kawamata N, Takeuchi S, Koike M, Asou H, Simpson JF, Koeffler HP. DPC 4/SMAD 4 in non-pancreatic tumors with frequent LOH 18q21 and in hematological malignancies. *Int J Oncol* 1997; **10**: 257–260.
- 301 Yodoi J, Uchiyama T. IL-2 receptor dysfunction and adult T-cell leukemia. *Immunol Rev* 1986; **92**: 135–156.
- 302 Uchiyama T, Kamio M, Kodaka T. Leukemic cells from some adult T-cell leukemia patients proliferate in response to interleukin-4. *Blood* 1988; **72**: 1182–1186.
- 303 Sodroski J, Rosen C, Goh WC, Haseltine W. A transcriptional activator protein encoded by the x-*Jor* region of the human T-cell leukemia virus. *Science* 1985; **228**: 1430–1434.
- 304 Migone TS, Lin JX, Cereseto A, Mulloy JC, O'Shera JJ, Leonard WJ. Constitutively activated Jak-STAT pathway in T cells transformed with HTLV-I. *Science* 1995; **269**: 79–83.
- 305 Wiedenfeld EA, Fernandez-Vina M, Berzofsky JA, Carbone DP. Evidence for selection against human lung cancers bearing p53 missense mutations which occur within the HLA A*0201 peptide consensus motif. *Cancer Res* 1994; **54**: 1175–1177.
- 306 Yanuck M, Carbone DP, Pendleton CD, Tsukui T, Winter SF, Minna JD, Berzofsky JA. A mutant p53 tumor suppressor protein is a target for peptide-induced CD8+ cytotoxic T-cells. *Cancer Res* 1993; **53**: 3257–3261.
- 307 Caron de Fromental C, May-Levine F, Mouriessé H, Lemerle J, Chandrasekaran K, May P. Presence of circulating antibodies against cellular protein p53 in a notable proportion of children with B-cell lymphoma. *Int J Cancer* 1987; **39**: 185–189.