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Homozygous deletion of the α- and β_1-interferon genes in human leukemia and derived cell lines

(tumor-suppressor genes/acute lymphoblastic leukemia/5'-methylthioadenosine phosphorylase/chromosome 9)

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ABSTRACT **The loss of bands p21–22 from one chromosome 9 homologue as a consequence of a deletion of the short arm [del(9p)], unbalanced translocation, or monosomy 9 is frequently observed in the malignant cells of patients with lymphoid neoplasias, including acute lymphoblastic leukemia and non-Hodgkin lymphoma. The α- and β_1-interferon genes have been assigned to this chromosome region (9p21–22). We now present evidence of the homozygous deletion of the interferon genes in neoplastic hematopoietic cell lines and primary leukemia cells in the presence or absence of chromosomal deletions that are detectable at the level of the light microscope. In these cell lines, the deletion of the interferon genes is accompanied by a deficiency of 5'-methylthioadenosine phosphorylase (EC 2.4.2.28), an enzyme of purine metabolism. These homozygous deletions may be associated with the loss of a tumor-suppressor gene that is involved in the development of these neoplasias. The relevant genes may be either the interferon genes themselves or a gene that has a tumor-suppressor function and is closely linked to them.**

Experimental evidence obtained during the past few years indicates that the genomes of various tumor cells contain mutated oncogenes that are dominant at the cellular level. Data from other experimental systems support the existence of a different class of oncogenes, which are recessive at the cellular level. Retinoblastoma and Wilms tumor are the prototypic biological models for the study of such oncogenes. The identification of individuals who had congenital deletions of chromosome 13 or 11, and who were predisposed to develop retinoblastoma or Wilms tumor, respectively, provided the initial evidence that the loss of function of dominant tumor-suppressor genes may be involved in the development of these tumors (1, 2). Similar mechanisms have been implicated recently in the pathogenesis of other tumors, including embryonal rhabdomyosarcoma (42), small-cell lung carcinoma (43), renal (44) and colorectal (45) carcinoma, meningioma (46), and acoustic neuroma (47). Although these mechanisms have not been well documented in the hematological malignant diseases, the high frequency of specific chromosome loss or deletion observed in a number of leukemias suggests that these genetic mechanisms may be important in the pathogenesis of these disorders.

In the lymphoid neoplasias, the loss of band p22 from one chromosome 9 homologue as a consequence of a deletion of the short arm [del(9p)], unbalanced translocation, or monosomy 9 is frequently observed in the malignant cells of patients who have acute lymphoblastic leukemia (ALL) or non-Hodgkin lymphoma (3–6). The identification of a specific chromosomal deletion involving 9p in these disorders suggests that a dominant tumor-suppressor gene may be located at 9p22. In this regard, the α- (*IFNA*) and β_1- (*IFNB1*) interferon (*IFN*) genes have been assigned to the affected chromosome region (9p21–22) (7, 8). In addition to their antiviral effects, IFNs have an antiproliferative effect (9, 10) and antigen-modulation effects (11, 12) on several types of cells. Some of these actions are mediated through an autocrine feedback loop that is triggered by growth factors such as macrophage-colony-stimulating factor or platelet-derived growth factor (13, 14); therefore, the *IFNA* and *IFNB1* genes may behave as tumor-suppressor genes, and their deletion or inactivation could be associated with the initiation or progression of neoplastic disorders.

To evaluate whether the *IFN* genes are involved in the deletions of 9p, we have analyzed a number of leukemia cell lines and primary leukemia cell samples from patients with ALL. Our results indicate that homozygous deletion of the *IFN* genes can be detected in neoplastic hematopoietic cell lines and primary leukemia cells in the presence or absence of deletions that are detectable at the level of the light microscope. In these cell lines, the deletion of the *IFN* genes is accompanied by a deficiency of 5'-methylthioadenosine phosphorylase (MTAP; 5'-methylthioadenosine:orthophosphate methylthioribosyltransferase, EC 2.4.2.28), an enzyme of purine metabolism.

MATERIALS AND METHODS

Cell Lines. The cell lines used in this study were derived from patients who had hematologic malignancies; these lines were provided by the investigators who had established them or they were obtained from the American Type Culture Collection. The cell lines are listed in Table 1.

Preparation of DNA, Gel Electrophoresis, and Southern Transfers or Dot Blots. DNA was prepared (24), digested with restriction enzymes, and separated by electrophoresis in 0.8% agarose gel slabs. DNA was transferred from the gels to nylon membranes as described (24). For dot blots, the DNA was denatured with NaOH and loaded on nylon membranes by using a 96-well dot-blotting apparatus (Bethesda Research Laboratories) under vacuum.

DNA Probes and Hybridization. The following cloned DNA sequences were used in the Southern or dot blot hybridizations: pTR48, a partial cDNA clone of the transferrin receptor (*TFRC*) gene (25); pL-fA, an IFNA2 cDNA clone (21); and pHFb, an *IFNB1* cDNA clone (22). The inserts were isolated from the vector sequences by restriction endonucle-

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Abbreviations: IFN, interferon (italicized when referring to genes); *IFNA*, α-interferon genes; *IFNB1*, β_1-interferon gene; MTAP, 5'-methylthioadenosine phosphorylase; ALL, acute lymphoblastic leukemia.

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Table 1. Presence or absence of *IFN* genes and of MTAP activity in human leukemia cell lines

Cell lineage	Diagnosis*	Cell line	*IFNA*†	*IFNB1*†	MTAP	Cytogenetic abnormality of 9p
T-cell	ALL	SUP-T3	−	−	−‡	None§
	ALL	K-T1	−	−	−‡	del(9)(p12p22.1)§
	ALL	RPMI-8402	+	+	− [15]	None§
	ALL	CCRF-CEM	+h	+h	− [16]	inv(9)(p11q13), del(9)(p11p22)¶ [48]
	ALL	CCRF-HSB2	+	+	− [16]	None¶ [17]
	ALL	SUP-T14	+h	+h		del(9)(p22)‖
	ALL	MOLT-16	+	+		der(9)t(9;9)(p24;p11), dic(9;15)(p11;p11)§
	CLL	SKW3	+	+		None§
	LL	SUP-T1	+	+		None§
	ALL	SUP-T8	+	+	+‡	None§
	ALL	MOLT-4	+	+	+ [15, 16]	None§
Pre-B	ALL	RCH-ACV	+	+		None§
	ALL	CL697	+	+		None§
B-cell	CML-BC	BV173	+	+		None§
	BL	Raji	+	+	+ [15, 16]	Unknown
Null (non-T, non-B)	ALL	Reh	−	−	− [15]	None¶ [18]
	ALL	RS4;11	−	−	−‡	None§
	ALL	NALL-1	−	−	− [15]	−9,del(9)(p13p23)§
	ALL	SUP-B13	+	+		None§
Myeloid	CML-BC	K562	−	−	−‡ [15, 16]	der(9)t(9;9)(p13;q22), del(9)(p12p24)§ [19]
	AML	HL-60	+h	+h	+‡	del(9p) [20]

Numbers in brackets are reference numbers.

*ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; LL, lymphoblastic lymphoma; CML-BC, chronic myelogenous leukemia in blast phase; BL, Burkitt lymphoma; AML, acute myelogenous leukemia.

†Presence of *IFNA* and *IFNB1* was determined by hybridization of dot blots or Southern blots to a cDNA probe of the *IFNA2* gene (21) and to a cDNA probe of the *IFNB1* gene (22). Autoradiographic signal of intensity comparable to that of the controls; +h, autoradiographic signal approximately half as intense as that of the controls; −, no signal.

‡MTAP activity determined by us: negative (−) cell lines showed <10% of control MTAP activity, measured as 5′-methylthioadenosine decomposition per mg of protein per minute, on two determinations, when assayed by a radiochemical technique (23).

§Cytogenetic analysis performed by us.

¶Published karyotype reviewed by us.

‖S.D.S. and F. Hecht, unpublished work.

ase digestion followed by gel electrophoresis and were used as templates to prepare labeled probes with Klenow fragment of DNA polymerase I and [α-^{32}P]dCTP, after priming with random hexanucleotides (49). Hybridization and washing of the blots were as described (24).

Determination of MTAP Activity. MTAP activity was determined by a radiochemical technique (23). Blood mononuclear cells from normal donors, or from stored primary leukemia cells that were previously found to have normal activity, were used as controls. Cell lines were called negative (−) when they showed <10% of control MTAP activity, measured as 5′-methylthioadenosine decomposition per mg of protein per minute, on two determinations.

Cytogenetic Analysis. For cytogenetic analysis, cells from cultures in logarithmic-phase growth were processed by routine techniques (50). Chromosomal abnormalities are described according to the International System for Human Cytogenetic Nomenclature, 1985 (27).

RESULTS AND DISCUSSION

By using Southern and dot blot analysis of DNA from 21 human hematopoietic cell lines, we found 6 independent cell lines that have a complete deletion of the *IFNA* and *IFNB1* genes (Figs. 1 and 2). These cell lines, which include NALL-1 (28) (data not shown), K562 (26), RS4;11 (29), Reh (30), and SUP-T3 and K-T1 (31), are derived from hematopoietic neoplasms of myeloid and B- and T-lymphoid lineages (Table 1).

Although we detected complete loss of the *IFN* genes by molecular analyses, at least one chromosome 9 homologue in four of these cell lines, SUP-T3, K-T1, RS4;11, and Reh, appears normal at the cytogenetic level (Fig. 3 and Table 1). The cell lines K562 and NALL-1 have cytogenetic abnormalities of the short arms of both chromosome 9 homologues (Table 1). The presence of chromosome 9 short arms that appear normal at the light-microscope level in four of these cell lines implies that the deletion that includes the *IFN* genes is relatively small, probably less than 2000 kilobase pairs (an average chromosome band has been estimated to be 5000 kilobase pairs long).

Three of the 21 cell lines, CCRF-CEM, SUP-T14, and HL-60, have hemizygous deletions of the same genes, coincident with deletions of 9p that were detected by cytogenetic analysis.

The deletion of the *IFN* genes in these cell lines may have occurred either *in vivo* or *in vitro*, during the establishment and propagation of the cell lines. To clarify this issue, we attempted to examine samples of the primary leukemia cells from which these cell lines were derived. This was feasible only for the SUP-T3 cell line. DNA from a bone marrow sample, which was obtained at the time of diagnosis of ALL and which contained 95% blasts, was analyzed by DNA dot blot hybridization. The signal from this sample was ≈0.1 times as intense as that from the placental DNA used as a control (Fig. 4). This suggested that the majority of cells in the sample had a complete loss of the *IFNA* and *IFNB1* genes and that the deletions occurred in the primary neoplastic cells. In addition, there was a smaller population of normal cells that was responsible for the remaining weak signal, thereby

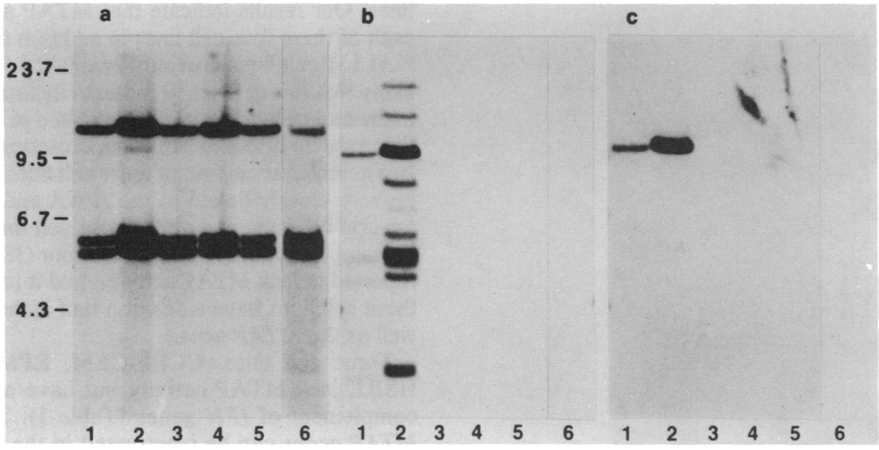

FIG. 1. Southern blot. DNA samples from human placenta and from various cell lines were digested with the restriction enzyme *Hin*dIII, electrophoresed, blotted, and hybridized successively to pTR48, a transferrin receptor (*TFRC*) probe (25) (*a*); pL-fA, an *IFNA2* cDNA probe (21) (*b*); and pHFb, an *IFNB1* cDNA probe (22) (*c*). Lane 1, normal human placenta (control); lane 2, BV173; lane 3, K562; lane 4, RS4;11; lane 5, K-T1; lane 6, SUP-T3. Position and sizes in kilobase pairs for *Hin*dIII-digestion fragments of λ phage DNA used as size markers are given at left.

demonstrating that the homozygous deletion of the *IFN* genes was not a constitutive defect of this patient. Our analyses of leukemia cells from additional patients have confirmed that deletion of the *IFN* genes can occur *in vivo*. Specifically, in a study of 42 patients who had ALL, it was found that 3 patients (7%) had homozygous deletions of the *IFNA* genes and 8 patients (19%) had hemizygous deletions of the same genes (M.O.D., M.M.L.B., and C. M. Rubin, unpublished results).

A deletion of the *IFNB1* gene has been reported in the fibroblast cell line Vero, which was derived from kidney cells of an African green monkey (32). We hybridized the human *IFNA* and *IFNB1* gene probes to Southern and dot blots of Vero DNA, and we found that the *IFNA* genes are also deleted (Fig. 2). The Vero cells were derived from normal fibroblasts; thus, it is likely that, in this case, the deletion of the *IFN* genes occurred *in vitro*.

The complete loss of the *IFN* genes in six neoplastic hematopoietic cell lines and in the primary leukemia cells from patients with ALL provides evidence for a homozygous deletion associated with leukemia. By analogy to deletions found in retinoblastoma (33, 34), this homozygous deletion may include a tumor-suppressor gene whose loss or inactivation plays a role in the initiation or progression of the neoplastic process. The deletions in four of the cell lines of our study were extremely small (they were not detected by light microscopy); therefore, the tumor-suppressor gene

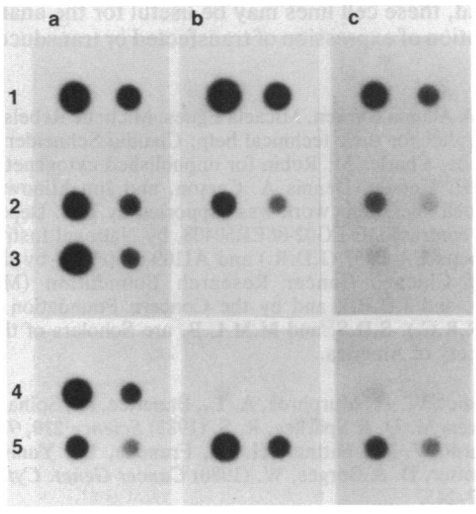

FIG. 2. Dot blots of DNA from human placenta and from various cell lines that were hybridized successively to the same probes listed in Fig. 1 (*a–c*). Samples of 1.0 and 0.2 μg of DNA were loaded in two spots for each sample. Row 1, human placenta; row 2, CCRF-CEM; row 3, Reh; row 4, Vero; row 5, COS1. Vero and COS1 are fibroblast lines derived from the African green monkey. DNA from the COS1 cell line was used as a control for the hybridization of the human probes to green monkey DNA.

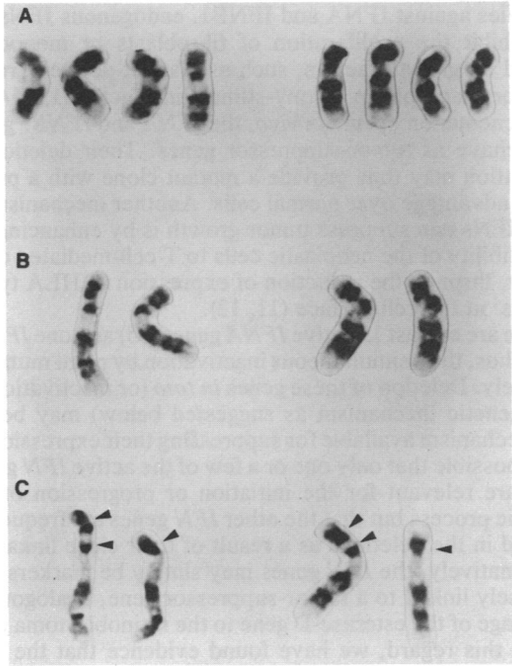

FIG. 3. Partial karyotypes illustrating chromosome 9 homologues from two metaphase cells from the SUP-T3 (*A*), RS4;11 (*B*), and K-T1 (*C*) cell lines. SUP-T3 is a tetraploid cell line and contains four chromosome 9 homologues. According to cytogenetic analysis, the chromosome 9 homologues in the SUP-T3 and RS4;11 cell lines have normal short arms [SUP-T3 has a t(7;9)(q35;q32), involving the long arms of the two chromosome 9 homologues on the right of each set of chromosomes], whereas the K-T1 cell line has an interstitial deletion of the short arm of one chromosome 9 homologue [del(9)(p12p22.1)]. Arrowheads in *C* indicate the breakpoints of the deletion on the normal homologue (left) and the breakpoint junction of the rearranged homologue (right).

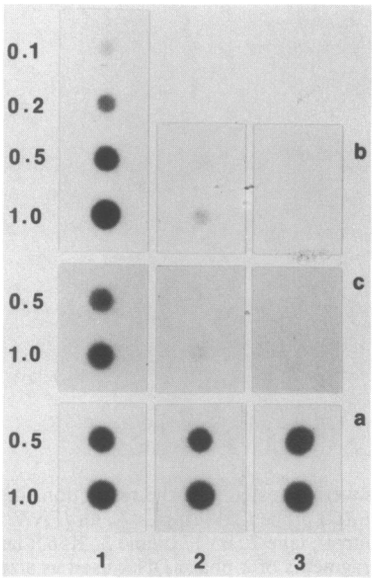

FIG. 4. Dot blot of DNA from normal human placenta (column 1), a bone marrow sample of the leukemia patient from whom the SUP-T3 cell line was derived (column 2), and the SUP-T3 cell line (column 3). Amounts of DNA, in μg, for each row of dots are given at left. This blot was hybridized successively to *TFRC* (*a*), *IFNA2* (*b*), and *IFNB1* (*c*) probes as described for Figs. 1 and 2.

must be very closely linked to, if not within, the *IFN* gene cluster.

IFNs added to the culture medium inhibit the proliferation of many types of cells (9, 10). As shown by the effect of antibodies against IFNA and IFNB1, endogenous IFNs can also inhibit the proliferation of fibroblasts or monocytes induced by growth factors, such as platelet-derived growth factor or macrophage colony-stimulating factor (13, 14). If this phenomenon occurs *in vivo*, the *IFNA* and *IFNB1* genes may behave as tumor-suppressor genes. Their deletion or inactivation may thus provide a mutant clone with a proliferative advantage over normal cells. Another mechanism by which IFNs can suppress tumor growth is by enhancing the susceptibility of the neoplastic cells to T-cell-mediated cytotoxicity, through the induction of expression of HLA type I antigens on the cell surface (11, 12).

There are at least 15 active *IFNA* genes (35) and one *IFNB1* gene. Thus, their simultaneous inactivation by point mutation is unlikely. Deletion of these genes *in toto* (or inactivation by an epigenetic mechanism as suggested below) may be the only mechanism available for suppressing their expression. It is also possible that only one or a few of the active *IFN* genes on 9p are relevant for the initiation or progression of the leukemic process but that the other *IFN* genes are frequently included in the deletions as a result of their close linkage.

Alternatively, the *IFN* genes may simply be markers that are closely linked to a tumor-suppressor gene, analogous to the linkage of the esterase-D gene to the retinoblastoma gene (36). In this regard, we have found evidence that the *IFN* genes are not the only genes included in these deletions: a deficiency of an enzyme of purine metabolism, MTAP, has been reported in several neoplastic cell lines, including the human leukemia cell lines K562, Reh, and NALL-1 (15, 16), as well as in samples of primary neoplastic cells from four patients who had leukemia (3, 37) and in those from three patients who had solid tumors (38). The human gene for MTAP has been assigned to 9pter–q12 by analysis of somatic cell hybrids (39).

To examine the relationship of MTAP activity to the submicroscopic deletions of 9p that we detected, we measured the MTAP activity in the RS4;11, SUP-T3, K-T1, and Vero cell lines. Our results indicate that MTAP activity is absent from each of these four cell lines in addition to the K562, Reh, and NALL-1 cell lines previously reported (15, 16) (Table 1). It is likely that this deficiency is due to inclusion of the *MTAP* gene in the molecular deletion that resulted in loss of the *IFN* genes. Since the 9p deletions are submicroscopic in at least one of the two homologues in four of these cell lines, the *MTAP* gene must be very closely linked to the *IFNA* and *IFNB1* gene cluster. Several other human and mouse cell lines (15, 16), including hematopoietic (3, 37) and solid tumor (38) cell lines, have been reported to lack MTAP activity, and it is possible that some of these cell lines have a deletion that includes the *IFN* genes as well as the *MTAP* gene.

Three cell lines, CCRF-CEM, RPMI-8402, and CCRF-HSB2, lack MTAP activity but have at least one complete complement of *IFN* genes (Table 1). This implies that the *MTAP* genes can be inactivated in the absence of complete deletion of the *IFN* genes. Nonetheless, in these cell lines, both the *IFN* genes and the *MTAP* gene may have been functionally inactivated by an epigenetic mechanism. In this regard, it has been reported that CCRF-CEM and RPMI-8402 cells do not produce IFNA after induction with Sendai virus (40). It has been reported that other T-cell leukemia cell lines fail to produce IFNA following induction; thus, this phenomenon may be a property of cells of this lineage, rather than of transformed cells. CCRF-HSB2 cells do not produce either IFNA or IFNB1 after induction with Sendai virus (41). Thus, functional inactivation of the complete *IFN* gene cluster and the *MTAP* gene may occur in human neoplasias through mechanisms other than the homozygous deletions described here.

Some of the cell lines that have hemizygous or homozygous loss of the *IFN* genes, or are deficient in the expression of these genes, are frequently used as a source of normal myeloid or lymphoid cells for a variety of investigations. The lack of *IFN* gene expression in these cells may critically affect the results of experiments that involve control of surface antigen expression, susceptibility to viral infection, control of cell proliferation or differentiation, and immune effector or target function. Therefore, caution must be used and the lack of *IFN* gene expression must be considered in the interpretation of the results of such investigations. On the other hand, these cell lines may be useful for the analysis of the regulation of expression of transfected or transduced *IFN* genes.

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