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Expression of Cadherin-Catenin Complexes in Human Leukemia Cell Lines[1]

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Cadherins are Ca^{2+}-dependent cell-cell adhesion molecules, and are involved in the formation and maintenance of the histo-architecture. Using cultured human leukemia cell lines (adult T cell leukemia and thymus-derived lymphoma cell lines), we obtained evidence that cadherins and catenins are expressed in these cell lines but not in normal leukocytes. Immunoblot analysis of cells using a pan-cadherin serum, directed against the conserved carboxyl-terminus of cadherins, revealed a major band of 130 kDa and a minor one of 135 kDa. The 130 kDa cadherin was also recognized by anti-N-cadherin antibodies. A human N-cadherin cDNA probe hybridized to a 4.3 kb mRNA isolated from cells immunologically positive for N-cadherin. Sequencing of the cDNA fragments isolated from the cells revealed a N-cadherin sequence. Cell surface expression of N-cadherin was confirmed by indirect immunofluorescence staining of the cells. Immunoblot and Northern blot analyses also revealed the presence of α-catenin, β-catenin, and γ-catenin (plakoglobin) in these cell lines. Immunoprecipitation with anti-N-cadherin antibodies and subsequent immunoblot analysis with anti-catenin antibodies revealed that N-cadherin is associated with α- and β-catenins, a prerequisite for cadherins to be functional. These results suggest an important role of the cadherin-catenin complexes in the behavior of the leukemia cells.

Key words: ATL, N-cadherin, α-catenin, β-catenin, leukemia.

Cadherins comprise a family of structurally and functionally related molecules that mediate Ca^{2+}-dependent cell-cell interactions in a homophilic manner (*1*). They are subdivided into several groups, such as classical cadherins and protocadherins, according to their primary structures (*2*). The classical cadherins, to which E-, P-, and N-cadherins belong, are highly conserved transmembrane glycoproteins with similar domains for homophilic binding, Ca^{2+}-binding, and interaction with intracellular proteins (*2*). Cadherins have been shown to play major roles during morphogenesis and in the maintenance of tissue structures (*3*). Most of these cadherin-dependent interactions are homophilic as well as homotypic, but recent experiments showed that cadherins can also be involved in heterotypic and even heterophilic interactions (*4-6*).

The cytoplasmic domain of cadherins interacts with three molecules called catenins (α, β, and γ), and the complex seems to be associated with cortical actin filaments (*7, 8*). This interaction between cadherins and catenins is essential for cadherin-mediated adhesion and association of the complex with the cytoskeleton (*9-11*). Recent *in vitro* and *in vivo* analyses revealed that β-catenin or γ-catenin binds directly to the cytoplasmic domain of cadherins, while α-catenin binds directly to β-catenin or γ-catenin (*12-16*).

During metastasis, a carcinoma cell must break away from the primary tumor, move into the surrounding stroma, invade the lymphatic or vascular circulation, and then start to grow at a secondary site. It is likely that an early, critical step in metastasis for an invading cell is detachment from neighboring cells. Since E-cadherin binds adjacent epithelial cells together, it is hypothesized to play a role in suppressing the invasive phenotype. Reduced, absent or disorganized expression of E-cadherin has been described in carcinomas, including gastric, head and neck, bladder, prostate, colon, and breast ones (*17-24*). Experiments involving *in vitro* model systems have revealed that cells in which E-cadherin is not expressed or inhibited are more invasive than ones with normal cadherin activity (*25-27*). Reintroduction of the E-cadherin gene into invasive cancer cells that were E-cadherin-negative resulted in a less invasive phenotype (*28*). Therefore, it has been proposed that, in addition to its role in development, E-cadherin may play a role as an "invasion suppressor gene" in cancer progression.

N-Cadherin (neural cadherin) is another member of the cadherin family and has a relative molecular weight of about 130 kDa (*1*). The expression of N-cadherin occurs in

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Abbreviations: ATL, adult T cell leukemia; bp, base pair(s); GST, glutathione S-transferase; MBP, maltose-binding protein; PAGE, polyacrylamide gel electrophoresis; PBS, Dulbecco's phosphate-buffered saline; PCR, polymerase chain reaction; RT, reverse transcription; SDS, sodium dodecyl sulfate.

neural tissues as well as in many kinds of nonneural tissues, such as mesenchymal tissue. Its expression pattern has been shown to be associated with a variety of morphogenetic events (*3*). The role of N-cadherin in neural tissues has been studied extensively. For example, N-cadherin has been implicated in the histogenesis of neural retina, and in the growth and guidance of axons during nervous system development (*29, 30*).

Since cadherins are known to be morphoregulatory molecules during embryonic development and are important in the maintenance of tissue integrity, there have been a few reports on the expression and involvement of cadherins in cells of hematopoietic lineage (*4, 5, 31*). In the present paper, we present evidence that cadherins and catenins are expressed in certain leukemia cell lines but not in normal leukocytes.

MATERIALS AND METHODS

Cell Lines—A human transitional carcinoma cell line, BOY (*32*), was cultured as described previously (*16*). The adult T cell leukemia (ATL) cell lines, Oh13T, F6T, K3T, S1T, and Su9T01, were established in our laboratory (*33*). ATL cell lines, a human premyelocytic leukemia cell line, HL60, human thymus-derived cell lines, Molt-4F and CEM, and human Burkitt lymphoma cell lines, Raji and EB3, were maintained in the same way (*33*). Fresh leukocytes from healthy volunteers were also used.

cDNA Clones—cDNA clones for human α-catenin, β-catenin, and γ-catenin have been described (*16*). The cDNA clone for human N-cadherin was isolated by means of reverse transcription-polymerase chain reaction (RT-PCR) from cDNA of BOY cells as described previously (*16*). Two oligonucleotides, N-1 (5′GACTGGGTCATCCCTCCAAT) and N-2 (5′TGGCTGTGTTTGAAAGGCCA), were synthesized and used as PCR primers. The N-1 primer corresponds to the published sequence of human N-cadherin cDNA (GenBank accession number, X57548) at nucleotides 1-20, and the N-2 primer comprises a sequence complementary to the cDNA at nucleotides 603-622. The reaction mixture was subjected to 30 cycles of denaturation (93°C, 1 min), annealing (56°C, 2 min), and extension (72°C, 3 min). The PCR product of the expected size (622 bp) was purified by agarose gel electrophoresis and then cloned into the *Sma*I site of the Bluescript KS(+) vector (Stratagene). The cDNA was sequenced on both strands as described previously (*34*). Cloning and sequencing of the N-cadherin cDNA fragments from human leukemia cells was performed in the same way.

Antibodies—Monospecific antibodies against the carboxyl-terminal part of γ-catenin were described previously (*16*). The full-length α-catenin and β-catenin, and the amino-terminal part of mature N-cadherin (residues 1-207) were expressed as fusion proteins with maltose-binding protein (MBP) or glutathione S-transferase (GST), and used as antigens or ligands for antibody purification, respectively. Thus, antisera were prepared by immunizing rabbits with N-cadherin, α-catenin, or β-catenin expressed in *Escherichia coli* as a fusion protein with MBP, and purified as described previously (*16*). Each antibody was purified by affinity chromatography on Sepharose coupled with either N-cadherin, α-catenin, or β-catenin, which were expressed as fusion proteins with GST as described previously (*16*). A monoclonal antibody against human E-cadherin (HECD-1) was obtained from Takara, and the monoclonal antibody against N-cadherin (GC4) and anti-pan cadherin antibodies were from Sigma.

Northern Blotting—Total RNA was isolated from cells by the acid guanidine isothiocyanate-phenol-chloroform extraction method (*35*). Total RNA (20 μg) was fractionated by electrophoresis on a 1% agarose gel, and then blotted onto a nylon membrane (Amersham), and probed with the N-cadherin, α, β, or γ-catenin cDNA fragments radiolabeled by the random priming method (*36*). The hybridization and washing conditions were as described previously (*34*).

Other Analysis—Cells were labeled with [^{35}S]methionine as described previously (*7*). Immunoblotting and immunoprecipitation were carried out as described previously (*7*) except that a POD (peroxidase) immunostain set (Wako) or an ECL detection kit (Amersham) was used in immunoblot experiments. Immunofluorescence staining was performed as described previously (*34*) using GC4 (for the detection of N-cadherin) or HECD-1 (for the detection of E-cadherin). Cell aggregation assay was done as described before (*9*).

RESULTS

Cadherins are Expressed in Some Leukemia Cells—To determine the possible expression of cadherins in human leukemia cell lines, we examined these cells for cadherin expression by immunoblotting using a pan-cadherin serum, directed against the conserved carboxyl-terminus of the cadherins. A major band of 130 kDa and a minor one of 135 kDa were detected for some leukemia cells (Oh13T, F6T, K3T, Molt-4F, and CEM), but not for others (S1T, Su9T01, HL60, Jurkat, Raji, and EB3) or normal leukocytes (Fig. 1). Neither the 130 kDa band nor the 135 kDa one was stained by antibodies against E-cadherin, suggesting that neither is E-cadherin (data not shown). We then probed the membrane with antibodies against N-cadherin. As shown in Fig. 2A, the major band of 130 kDa of Oh13T cells was recognized by these antibodies. Thus the 130 kDa band of Oh13T cells is N-cadherin. With other cell lines no clear band was detected with the N-cadherin antibodies under the conditions used. The lower content of N-cadherin protein could be the reason for this. The presence of a yet not identified cadherin protein in these N-cadherin protein-negative cell lines has not been excluded.

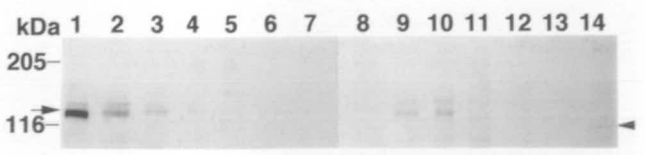

Fig. 1. Immunoblot analysis of human leukemia cell lines with pan-cadherin antibodies. Human leukemia cells were boiled in the SDS-PAGE sample buffer and then run on 8% polyacrylamide gels. After electrophoresis, the proteins were transferred to nitrocellulose membranes and reacted with a pan-cadherin antiserum. Cells analyzed: BOY (lane 1), Oh13T (lane 2), F6T (lane 3), K3T (lane 4), S1T (lane 5), Su9T01 (lane 6), HL60 (lane 7), Jurkat (lane 8), Molt-4F (lane 9), CEM (lane 10), Raji (lane 11), EB3 (lane 12), normal leukocytes (lane 13), and E-cadherin positive cell line, MCF-7 (lane 14). The arrow indicates the 130 kDa band. The arrowhead indicates the expected position of E-cadherin.

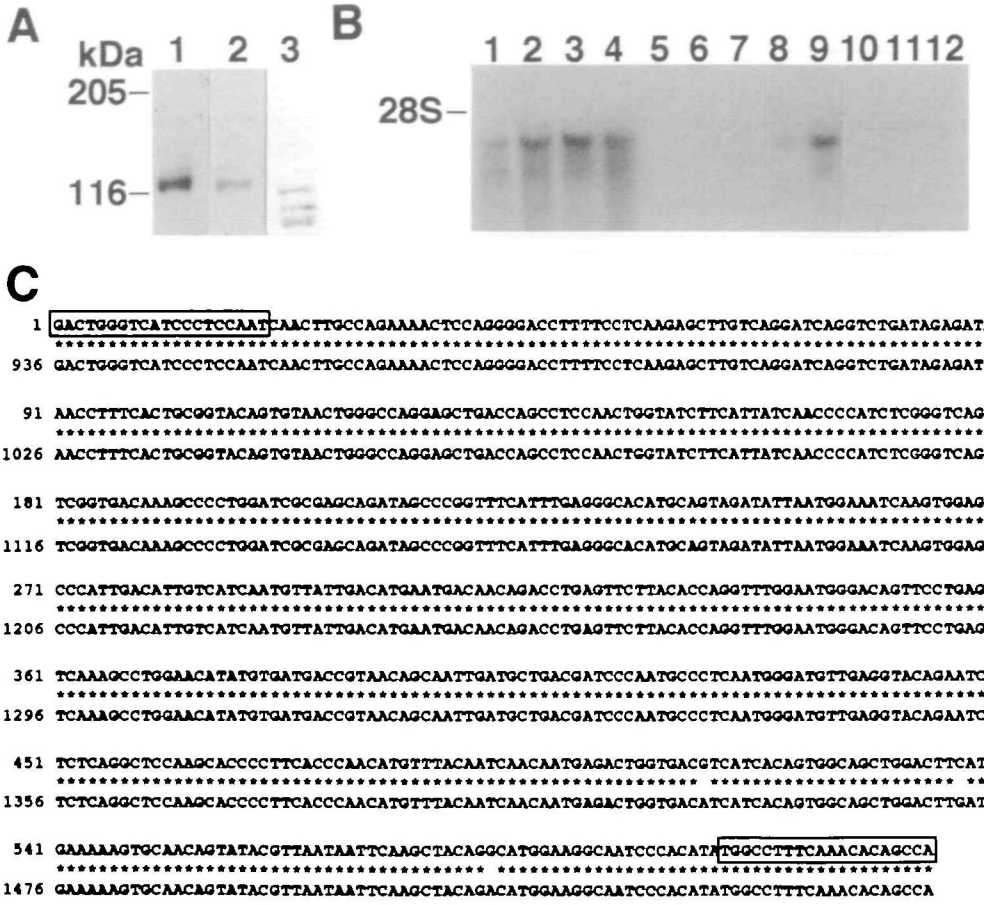

Fig. 2. **Expression of the N-cadherin protein (A) and mRNA in leukemia cell lines (B), and the sequence of N-cadherin cDNA from a leukemia cell line (C).** (A) Proteins from BOY (lane 1, positive control), Oh13T (lane 2), and MCF7 (lane 3, negative control) cells were subjected to immunoblot analysis with anti N-cadherin antibodies. (B) A single transcript of approximately 4.3 kb was detected, using a human N-cadherin cDNA probe, in total RNA isolated from the human leukemia cell lines. Cells analyzed: BOY (lane 1), Oh13T (lane 2), F6T (lane 3), K3T (lane 4), S1T (lane 5), Su9T01 (lane 6), HL60 (lane 7), Jurkat (lane 8), Molt-4F (lane 9), Raji (lane 10), EB3 (lane 11), and normal leukocytes (lane 12). (C) The nucleotide sequence of the N-cadherin cDNA from a leukemia cell line (Oh13T, upper lanes) was aligned with the published one (*44*). The former shows three nucleotide changes from the latter. The possible nucleotide changes during PCR amplification are not excluded.

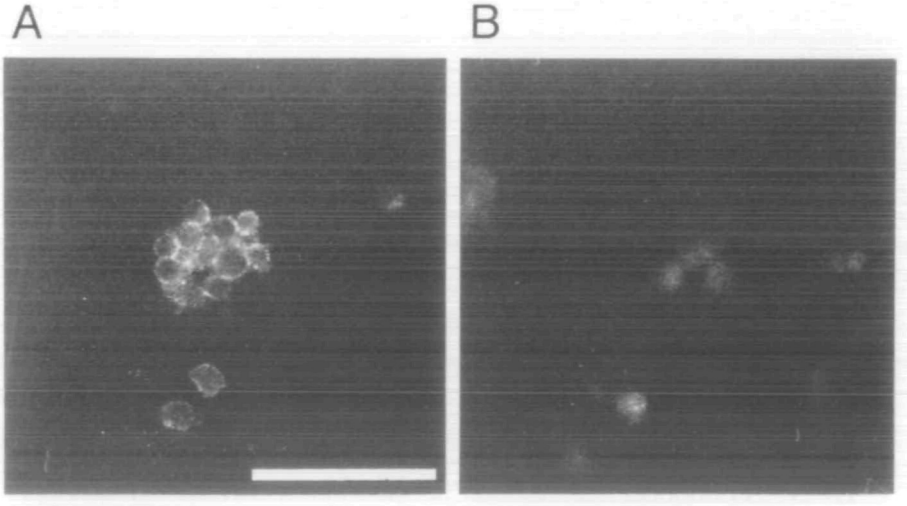

Fig. 3. **Immunofluorescence staining of human leukemia cell lines with N-cadherin antibodies, showing the cell surface expression of the protein.** Cells (Oh13T) were stained with either N-cadherin (A) or E-cadherin (B) antibodies followed by with FITC-conjugated anti-mouse IgG. Scale bar, 50 μm.

The expression of N-cadherin mRNA in these cadherin-positive cell lines was examined by Northern blot analysis using a N-cadherin cDNA as a probe. Positive hybridization was observed to a mRNA species of 4.3 kilobases in length in each cell line immunologically positive for cadherins, but not in cell lines negative for cadherins (Fig. 2B). Cloning and sequencing of cDNA fragments isolated from the N-cadherin-positive cell lines by RT-PCR using N-cadherin specific oligonucleotides revealed a N-cadherin sequence (Fig. 2C). These immunological and molecular biological data demonstrate the expression of N-cadherin in certain leukemia cell lines.

Cell surface expression of N-cadherin on Oh13T cells was assessed by indirect immunofluorescence staining of living cells with anti-N-cadherin antibodies. Cells positive for N-cadherin on immunoblot analysis were stained by the antibodies (Fig. 3A), but cells negative for N-cadherin, including peripheral blood leukocytes, were not (not shown). In contrast, anti-E-cadherin antibodies gave no staining (Fig. 3B).

Catenins are also Expressed in Leukemia Cell Lines— Since cadherins such as N-cadherin are associated with intracellular proteins called catenins, and complex formation with catenins is a prerequisite for the cell adhesive function of cadherins, we next examined whether or not these cell lines express catenins. Immunoblot analysis of the cadherin-positive cells revealed that they express α-catenin (not shown), β-catenin (Fig. 4A), and γ-catenin (not shown). Northern blot analysis with the respective cDNA probes confirmed the expression of mRNAs for α-catenin (not shown), β-catenin (Fig. 4B), and γ-catenin (not shown). To determine whether or not cadherins are indeed associated with catenins in these cell lines, we first obtained N-cadherin from lysates of Oh13T cells by immunoprecipitation with N-cadherin antibodies, and the collected material was subjected to immunoblotting with antibodies against α-catenin (not shown) and β-catenin (Fig. 4C). α-Catenin and β-catenin were coprecipitated by the N-cadherin antibodies together with N-cadherin. Therefore, N-cadherin in the leukemia cells was associated with catenins like N-cadherin in other cell types. Immunoprecipitation analysis of labeled cells with anti-β-catenin antibodies revealed bands of 130 and 135 kDa together with β-catenin and α-catenin bands (Fig. 4D, lane 2). When the materials collected by immunoprecipitation with anti-β-catenin antibodies were blotted with pan-cadherin antiserum, a major band of 130 kDa corresponding to N-cadherin and a minor one of 135 kDa were detected (Fig. 4D, lane 3). Therefore not only N-cadherin but also the 135 kDa cadherin in the leukemia cells seemed to be complexed with catenins.

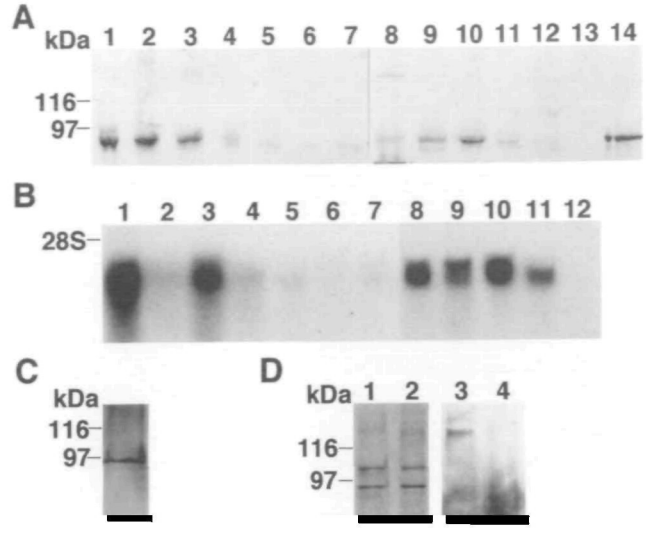

Fig. 4. **Expression of β-catenin in leukemia cell lines and its association with N-cadherin.** Immunoblot (A) analysis of human leukemia cell lines with affinity-purified antibodies against β-catenin. Cells analyzed: BOY (lane 1), Oh13T (lane 2), F6T (lane 3), K3T (lane 4), S1T (lane 5), Su9T01 (lane 6), HL60 (lane 7), Jurkat (lane 8), Molt-4F (lane 9), CEM (lane 10), Raji (lane 11), EB3 (lane 12), normal leukocytes (lane 13), and MCF-7 (lane 14). Northern blot (B) analysis of the cell lines with a β-catenin probe. Cells analyzed: BOY (lane 1), Oh13T (lane 2), F6T (lane 3), K3T (lane 4), S1T (lane 5), Su9T01 (lane 6), HL60 (lane 7), Jurkat (lane 8), Molt-4F (lane 9), Raji (lane 10), EB3 (lane 11), and normal leukocytes (lane 12). Immunoblot analysis of proteins associated with N-cadherin (C). Proteins precipitated by N-cadherin antibodies together with N-cadherin from Oh13T cells were subjected to immunoblot analysis with antibodies against β-catenin (C). Immunoprecipitation and immunoblot analysis of proteins associated with β-catenin in leukemia cells (D). BOY (lane 1) and Oh13T (lane 2) cells labeled with [^{35}S]methionine were subjected to immunoprecipitation by anti-β-catenin antibobies. Proteins precipitated by anti-β-catenin antibodies (lane 3) or control antibodies (anti-GST) (lane 4) from Oh13T cells were probed with pan-cadherin antiserum.

DISCUSSION

The combination of biochemical and molecular biological analyses revealed that at least two types (130 and 135 kDa) of cadherins are expressed in some leukemia cell lines, but not in normal leukocytes (Table I). We identified one of them as N-cadherin. The 135 kDa cadherin has not been identified, but it can be classified as a classical cadherin because it is recognized by pan-cadherin antiserum and it is associated with β-catenin. Furthermore, α-, β-, and γ-catenins are also expressed in these cell lines. Since complex formation with catenins is a prerequisite for cadherins to be active, we assume that the cadherins expressed in the leukemia cell lines could be functionally active. We tested whether the cadherin-positive cells show cadherin-dependent cell-cell adhesion using a standard cell aggregation assay (*3*). Although the cadherin-positive cells form clumps or aggregates during cell culture, they did not show aggregating activity under the conditions used (data not shown). At present we do not know the reason why we could not detect aggregating activity in these cells. Since the extent of cell aggregation in the aggregation assay with a shearing force depends on the amounts of cadherins expressed on cells (*37*) and the amounts of cadherins expressed on the leukemia cells were less than one third of that on the cells (BOY) with the activity (Fig. 1, lanes 1, 2, and 3, and Fig. 2A, lanes 1 and 2), the lower amounts of cadherins on these cells could be a possible explanation. Modifications such as tyrosine phosphorylation of catenins, found in *src*-transformed cells (*38*), could be an another explanation. Our preliminary experiments, however, suggest this less likely because we could not detect any significant degree of tyrosine phosphorylation of catenins in these cells.

TABLE I. **Expression of cadherin-catenin components in leukemia cell lines.**

	ATL					T cell leukemia			B cell leukemia		Premyelocytic leukemia	Normal
	Oh13T	F6T	K3T	S1T	Su9T01	Jurkat	Molt-4F	CEM	Raji	EB3	HL60	lymphocyte
130 and 135 kDa-cadherin	+	+	+	−	−	±	+	+	−	−	−	−
N-Cadherin Northern	+	+	+	−	−	+	+	ND	−	−	−	−
N-Cadherin Northern												
α-Catenin												
Western	+	+	+	−	−	−	+	−	−	−	−	−
Northern	+	+	+	±	±	+	+	ND	−	−	−	−
β-Catenin												
Western	+	+	+	±	±	+	+	+	±	±	±	−
Northern	+	++	+	±	±	++	++	ND	++	++	±	−
γ-Catenin												
Western	+	+	+	−	−	+	−	−	+	−	+	−
Northern	+	+	+	−	−	+	−	ND	++	−	+	ND

ND, not done; ++, strongly positive; +, positive; ±, weakly positive; −, negative.

Cadherins are Ca^{2+}-dependent cell-cell adhesion molecules, and are involved in the formation and maintenance of the histo-architecture (*3*). In that sense, our finding that cadherin-catenin complexes are expressed in human leukemia cell lines is rather unexpected because leukemia cells do not form tissue structures. Therefore the physiological meaning of the expression of cadherins and catenins in the leukemia cell lines remains to be determined. Under the conditions used we detected cadherins and catenins in three ATL cell lines and three T cell leukemia ones, but not in two B cell leukemia ones or one myeloid one. Normal leukocytes were also negative for these cadherins. Although the number of cell lines analyzed was limited, it is interesting that all the cadherin-positive cell lines are T cell leukemia-lymphoma ones. The two ATL cell lines, however, were negative for cadherins. Therefore we cannot simply link cadherin expression to the pathology of T cell leukemia. Tax is known to induce the expression of many cellular genes (*39*). The expression of N-cadherin and the 135 kDa cadherin in ATL cell lines, however, can not be correlated with the expression of the Tax protein since only two (F6T and K3T) of the cell lines expressing the cadherins were positive for the Tax protein. Further studies are needed for the use of cadherins and catenins as markers defining certain types of leukemia cells.

Heterotypic interactions involving the homophilic binding of E-cadherin have been observed between keratinocytes and Langerhans cells, a population of leukocytes in the epidermis, and between thymic epithelial cells and immature thymocytes (*4, 5*). E-Cadherin has been proposed to be important in the localization and persistence of Langerhans cells in the epidermis by facilitating cell adhesion between these cells. Frequent invasion of ATL cells into the epidermis has been reported (*40, 41*). Although the possible involvement of a variant type of sialyl Lewis X antigen expressed on ATL cells has been reported (*42*), the mechanism underlying the frequent skin involvement is unknown. ATL cells are often found in mesenchymal tissues of the skin, where N-cadherin is expressed. Our demonstration of N-cadherin expression in some ATL cells raises the possibility that the homophilic binding of N-cadherin on ATL cells and mesenchymal cells may be involved in the process. Although the cadherin-positive cells showed no aggregating activity, some qualitative or quantitative changes including up-regulation of cadherin expression on these cells, which might induce strong homophilic binding activity, may occur under certain micro-environments.

Although cadherin-positive cells contain a rather large amount of N-cadherin mRNA compared with BOY cells, the amount of protein expressed in the cells is not as high as that expressed in BOY cells. This might suggest the occurrence of post-transcriptional regulation in these cell lines.

Some cadherin-negative cell lines, such as Raji cells, express catenins. Furthermore, even in cadherin-positive cells the relative amount of β-catenin seemed to be much higher than those of cadherins (Fig. 1, lanes 1, 2, and 3, and Fig. 4A, lanes 1, 2, and 3). β-Catenin in leukemia cells may participate in cellular functions other than cell adhesion. In *Drosophila*, Armadillo, a homologous protein of β-catenin and γ-catenin, mediates a step in the signaling cascade that is initiated by Wingless, a *wnt* protein homologue, and leads to the establishment of the anterior-posterior polarity of the fly segments (*43*). Therefore it would be interesting to determine what kinds of complexes together with β-catenin are formed in these cells.

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