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The N-terminal propiece of interleukin 1α is a transforming nuclear oncoprotein

(cytokine/mesangial cells/oncogene)

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ABSTRACT **Interleukin 1α (IL-1α) is a pleiotropic cytokine involved in the immune response, inflammatory processes, and hematopoiesis, and acts as a mitogen for several malignant cell types, including acute leukemia and Kaposi sarcoma cells. These diverse activities have been exclusively attributed to the plasma membrane receptor-binding, 17-kDa C-terminal component (mature IL-1α) that results from proteolytic processing of the 31- to 33-kDa precursor protein. No biologic function has been ascribed to the unusually large, 16-kDa N-terminal propiece formed as a result of proteolytic processing of IL-1α. We report that the IL-1α N-terminal propiece is concentrated by means of a nuclear localization sequence within the nuclei of both transfected and leukemic cell lines. Overexpression of this component in glomerular mesangial cells, a model perivascular myofibroblast cell type capable of IL-1α synthesis and processing, results in malignant transformation to a spindle cell-type tumor. The functionally bipartite nature of the IL-1α precursor represents a unique combination of the C-terminal, classical cytokine and an N-terminal nuclear oncoprotein. These findings suggest that nuclear transport of the IL-1α N-terminal component may represent a critical component in the transformation of IL-1α-producing cells in the bone marrow or the perivascular area to a malignant phenotype.**

Peptide growth factors are crucial mediators of a cell's ability to grow and differentiate into specialized tissues. When these factors' tightly controlled synthetic rates are disrupted, however, overproduction can result in autocrine stimulation of growth and eventual malignant transformation. Several growth factors are known to function as oncogenes under these circumstances, including platelet-derived growth factor, the fibroblast growth factors, epidermal growth factor, and transforming growth factor α. These oncogenic factors all act via autocrine stimulation of the cell by undergoing secretion and thereafter binding to plasma membrane signaling receptors found on the surface of the same or neighboring cells (1–4). Alternatively, an intracellular pathway has been proposed whereby growth factor binding to newly synthesized signaling receptors in transit within the endoplasmic reticulum results in abnormal stimulation of second messenger pathways (5). To date, no peptide growth factors have been identified as strictly nuclear-acting oncoproteins, analogous to c-fos or c-myc, which transform cells as a consequence of direct DNA interactions or altered transcriptional regulation.

Interleukin 1α (IL-1α) is a central mediator of the immune and inflammatory response and also has a critical regulatory role in early hematopoiesis (6). Furthermore, IL-1α acts as an autocrine growth stimulant of several malignant cell types, including gastric carcinoma cells, B-lineage lymphoblasts, acute myelogenous leukemia cells, and the spindle cells of Kaposi sarcoma (7–10). Translated as a biologically active 31- to 33-kDa precursor protein, IL-1α is proteolytically processed by the calcium-activated neutral protease calpain into a 16-kDa N-terminal propiece and a 17-kDa C-terminal component, which is the extracellularly active region generally referred to as "mature" or processed IL-1α. The binding site for the plasma membrane-localized IL-1 receptors (types I and II) occurs within the 17-kDa C-terminal segment of the molecule, and the diverse responses elicited by either the biologically active precursor or the processed IL-1α molecule have been exclusively attributed to interactions of the C-terminal region with IL-1 plasma membrane receptors (11, 12). In addition to initiation of intracellular signaling mechanisms, the receptor/C-terminal IL-1α complex can be internalized and transported to the nucleus, a process mediated by a nuclear localizing sequence in the receptor protein (13, 14).

No distinct biologic role for the N-terminal propiece has been defined, although several potentially significant post-translational modifications, including phosphorylation and myristyl acylation, occur within this region (15–17). The IL-1α precursor has properties more characteristic of an intracellular than a secretory protein as it lacks a conventional hydrophobic signal sequence, is not localized to the Golgi apparatus or endoplasmic reticulum, and is translated on cytoskeletal-associated rather than membrane-bound polyribosomes (18, 19). We have recently shown that, after the processing of the 31- to 33-kDa precursor, the IL-1α 16-kDa N-terminal propiece persists intracellularly within endotoxin-stimulated human monocytes (17). In addition, localization studies using region-specific antibodies raised against epitopes within the N- and C-terminal segments of IL-1α suggested a preferential nuclear concentration of the N-terminal propiece (19). Based on these observations, our hypothesis was that the IL-1α N-terminal propiece persists intracellularly after precursor processing and is subsequently targeted to the nucleus independently of pathways involving the plasma membrane receptor-bound C-terminal, mature IL-1α. In this report, we demonstrate that this targeting occurs and that cellular transformation of the perivascular mesangial cell (MC) to a spindle cell-type tumor is a direct consequence of this event.

MATERIALS AND METHODS

Cell Culture. Rat glomerular MC were prepared, characterized, and grown as previously reported in detail (20, 21).

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Abbreviations: ADH, alcohol dehydrogenase; HSA, human serum albumin; IL-1α, interleukin 1α; MC, mesangial cell(s); NLS, nuclear localization sequence; IL-1α33, 33-kDa precursor IL-1α.

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The transfection studies used several individually derived cell lines of these cultures. Human promyelocytic leukemia HL-60 cells were obtained from the American Type Culture Collection and maintained in RPMI 1640 medium supplemented with 10% fetal calf serum. Induction of IL-1α synthesis was performed by incubation for 24 hr in serum-free RPMI 1640 medium containing 10 nM phorbol 12-myristate 13-acetate (Sigma).

Soft agar assay of transfected MC was performed using 5×10^3 cell/ml plating agar (0.33% agar in DMEM/10% serum). Colonies were visible at 7 days and were photographed at day 14.

Nuclear Targeting Assay. A rhodamine–human serum albumin (HSA) conjugate was prepared according to Moore and Blobel (22) and coupled to the terminal cysteine residue of the IL-1α peptide (T_{76}NGKVLKKRRLSLSQC) using Sulfo-SMCC (Pierce). The nuclear targeting assay was performed as described (22). MC were cultured on fibronectin-coated glass coverslips and incubated for 5–15 min at 15°C with the nuclear transport mixture containing (in a final volume of 20 μl) 1 μl of 20 mg/ml BSA, 1 μl of 20 mM ATP, 1 μl of 100 mM phosphocreatine, 1 μl of 400 units/ml creatine phosphokinase, 0.5 μg of peptide conjugate, and 200 μg of oocyte lysate. The slips were rinsed, fixed in 3% paraformaldehyde, and mounted in Aquamount containing n-propyl gallate.

Construction of IL-1α Expression Vectors. The full-length cDNA for human IL-1α was obtained by PCR from a phorbol ester-induced HL-60 cDNA template and directionally subcloned into the expression vector pcDNA-1 (Invitrogen). The 16-kDa N-terminal IL-1α cDNA was prepared from the 33-kDa precursor IL-1α (IL-1α33) template using PCR primers designed to introduce a stop codon after bp 356 (site of calpain cleavage), followed by directional subcloning into pcDNA-1. For site-specific mutagenesis of the IL-1α nuclear localization sequence (NLS; $K_{82}K_{83} \rightarrow Thr_{82}Thr_{83}$), the N-terminal IL-1$\alpha$ cDNA was subcloned into pcDNA-AMP (Invitrogen), and double-stranded mutagenesis was performed with the U.S.E. Kit (Pharmacia). The N-terminal IL-1α/*Drosophila* alcohol dehydrogenase (ADH) fusion construct was prepared by a three-part ligation into *Bam*HI/*Xho*I-digested pcDNA-1 using the *Bam*HI/*Pst*I N-terminal IL-1α cDNA and a *Pst*I/*Xho*I cDNA encoding the open reading frame of *Drosophila* ADH. ADH histochemical stain was performed according to P. Martin *et al.* (23).

Transfections. Subconfluent MC cultures were transfected using Lipofectin (GIBCO) with use of the pcDNA constructs in a 8:1 ratio with the selection vector pRSVneo. Cells were selected with 800 μg/ml G418, single cell-subcloned, and screened for IL-1α protein expression using dot-blot analysis of cytosolic extracts. Patterns and quantities of integrated plasmids were determined by standard Southern blot analysis of isolated genomic DNA from the respective clones.

Antibody Preparation and Western Blot Analysis. Affinity-purified rabbit antibodies to epitopes within the N- and C-terminal regions of the IL-1α protein were prepared and characterized as reported (19). In brief, animals were immunized with keyhole limpet hemocyanin-coupled peptides corresponding to the IL-1α N-terminal sequence T_{76}NGKVLKKRRLSLSQC or the C-terminal sequence C_{167}DMGAYKSSKDDAKITV. IgG fractions were purified by DEAE-dextran and peptide affinity chromatography. The specificities of the affinity-purified antibodies were confirmed by pre-absorption studies with immunizing peptides and by Western blot analysis of stimulated human monocyte lysates (19).

Western blot analyses of control and IL-1α33-transfected MC cytosolic and nuclear extracts were performed. Control and transfected cells were lysed in buffer containing 0.5% Nonidet P-40 and sonicated. Nuclear pellets were recovered by centrifugation at $2000 \times g$, recentrifuged through 2 M sucrose cushions, and resuspended in 10 mM Tris·HCl, pH 7.4/2 mM $MgCl_2$/1 mM $CaCl_2$, containing 40 μg/ml DNAse I. After incubation for 30 min at room temperature, the pellets were washed twice in DNAse buffer, dissolved in 2% SDS, and recentrifuged. The extractable nuclear proteins in the supernatant were recovered. Cytosolic or nuclear extracts were electrophoresed on SDS/PAGE gels, transferred to nitrocellulose, and incubated sequentially with affinity-purified anti-N-terminal IL-1α IgG and biotinylated goat anti-rabbit IgG (Zymed), followed by incubation with ^{125}I-streptavidin and autoradiography.

Immunofluorescence Studies. Cells were grown on etched glass ccoverslips, washed, and fixed for 20 min in buffered 4% paraformaldehyde. After permeabilization for 90 sec in 0.1% Triton X-100, the cells were blocked in 5% goat serum and incubated overnight with 1 μg anti-C-terminal or anti-N terminal IL-1α IgG/ml PBS containing 0.1% BSA. Rinsed slips were subsequently incubated with 5 μg/ml biotinylated goat anti-rabbit IgG (Vector), washed, and incubated with 1:50 streptavidin/rhodamine (Molecular Probes), followed by mounting in glycerol-containing n-propyl gallate.

Tumorigenesis Analysis of N-Terminal IL-1α Transformants. Cells (1×10^6 per animal) from transfection controls, 31- to 33-kDa IL-1α and 16-kDa N-terminal IL-1α transfectant clones were injected intraperitoneally into groups of six athymic nu/nu mice (The Jackson Laboratory). All animals were killed after 4 weeks. Grossly visible tumor masses were excised, fixed in Bouin's solution, embedded in paraffin, and stained with hematoxylin/eosin.

RESULTS

The IL-1α 16-kDa N-terminal propiece contains one region with a high concentration of basic amino acids characteristic of many NLSs. To determine if this sequence alone, in the absence of additional IL-1α N-terminal propiece sequences, had the ability to directly mediate nuclear transport of a heterologous protein, a synthetic peptide containing the polybasic region (T_{76}NGKVLKKRRLSLSQC) was coupled to rhodamine-labeled HSA. This conjugate was applied to permeabilized rat MC in a reaction mix containing *Xenopus* oocyte cytosolic fractions that permit nuclear transport of NLS-containing proteins (22). NLS peptide-coupled HSA, but not HSA alone, rapidly (<5 min) concentrated in the nucleus (Fig. 1). Removal of a single positive charge within the polybasic region by myristyl acylation of K_{83} markedly reduced nuclear translocation (data not shown).

To determine whether the putative NLS functioned within the context of the IL-1α protein, the cDNAs coding for either

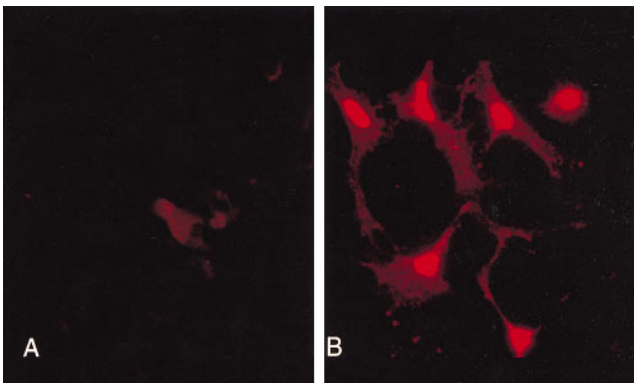

FIG. 1. Nuclear import assay to determine NLS activity. (*A*) Control consisting of permeabilized MC incubated with rhodamine-conjugated HSA alone. (*B*) Cells incubated with rhodamine-conjugated HSA coupled to the IL-1α NLS peptide sequence demonstrate intense nuclear immunofluorescence, consistent with active nuclear transport of the rhodamine/HSA reporter protein. (×350.)

the IL-1α 16-kDa N-terminal segment or the entire 33-kDa precursor were stably transfected into an established line of rat glomerular MC. These cells do not express significant levels of IL-1α under basal conditions, but are able to synthesize and fully process the molecule, with extracellular release of the C-terminal component, when stimulated by inflammatory mediators (24, 25). Single cell clones were selected, and IL-1α intracellular localization was studied using species-specific, affinity-purified antibodies targeted to epitopes within the N- or C-terminal portions of the human IL-1α precursor. MC transfected with the N-terminal IL-1α propiece exhibited marked nuclear staining when probed with the N-terminal-specific antibody (Fig. 2*A*). Site-specific mutagenesis that converted the NLS region $K_{82}K_{83}$ residues to $Thr_{82}Thr_{83}$ resulted in abrogation of the N-terminal propiece nuclear targeting (Fig. 2*B*). Cells expressing the entire IL-1α33 also showed nuclear staining with the N-terminal-specific antibody (Fig. 2*C*). In contrast, the C-terminal-specific antibody stained primarily the cytosol, with minimal nuclear staining (Fig. 2*D*). These differential distributions suggest that proteolytic processing of the IL-1α precursor is required for nuclear targeting and that it is the IL-1α 16-kDa N-terminal propiece alone, and not the entire 33-kDa precursor, that is specifically targeted to the nucleus. The same pattern of differential staining for the N- and C-terminal IL-1α segments was also seen in phorbol ester-stimulated human promyelocytic HL-60 leukemia cells (Fig. 3), indicating that selective targeting of the N-terminal IL-1α propiece also occurs in a nontransfected cell type capable of endogenous IL-1α synthesis and processing (26). A similar pattern of differential staining was also detected with phorbol ester-stimulated human promonocytic U937 cells (data not shown).

To confirm the findings of the immunofluorescence studies, Western blot analysis was performed on cytosolic and nuclear extracts of control and IL-1α33-transfected cells. As shown in Fig. 4, IL-1α33-transfected cells contained the 33-kDa precursor protein only in the cytosolic fraction, along with a small quantity of the N-terminal component. In contrast, only the 16-kDa IL-1α N-terminal component could be detected in the nuclear extracts of transfected cells. Isolated C-terminal component was not detected in either fraction (not shown).

Single cell clones of the IL-1α N-terminal propiece-transfected cells exhibited markedly altered culture morphology. Control MC stably transfected with the expression vector alone had the typical growth patterns of this cell type, with growth as nonorganized, rapidly migratory, shingle-shaped cells (Fig. 5*A*). In contrast, multiple single cell clones that expressed high levels of the IL-1α N-terminal propiece uniformly developed a population of elongated, refractile cells that formed a highly organized reticular network (Fig. 5*B*). A second distinct population of larger, rounded cells with more abundant cytoplasm was concentrated in the interstices formed by the elongated cell type. Repeated single cell subcloning always resulted in cultures expressing both distinctive

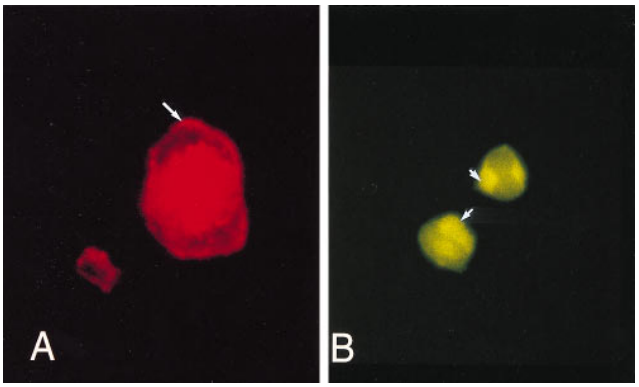

FIG. 3. Differential distribution of the N- and C-terminal segments of the IL-1α precursor in phorbol ester-induced HL-60 human promyelocytic leukemia cells. (*A*) Induced cells exhibit intense nuclear staining, as well as plasma membrane staining (white arrow) when probed with the N-terminal-specific antibody. (*B*) The C-terminal-specific antibody yields only cytosolic staining (arrows). (*A*, ×700; *B*, ×270.)

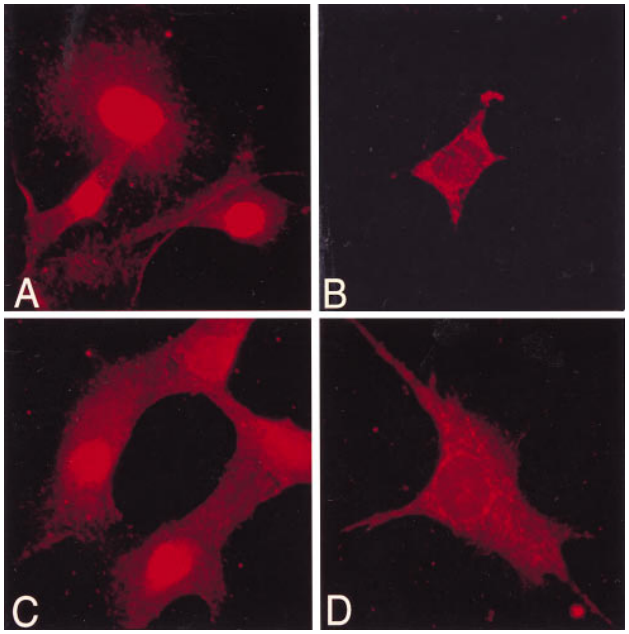

FIG. 2. Differential distributions of N- and C-terminal IL-1α segments in stably transfected MC. (*A*) Intense nuclear staining in cells transfected with the 16-kDa N-terminal IL-1α propiece, stained with N-terminal-specific antibody. (*B*) Transfected cells expressing $K_{82}K_{83} \rightarrow Thr_{82}Thr_{83}$ mutated N-terminal IL-1α propiece reveal intense cytosolic staining, with minimal nuclear staining using N-terminal-specific antibody. (*C*) Transfected cells expressing the 31- to 33-kDa IL-1α precursor exhibit bright nuclear staining with the N-terminal-specific antibody; with the C-terminal-specific antibody (*D*), staining is limited to the cytosol. (*A–D*; ×750.)

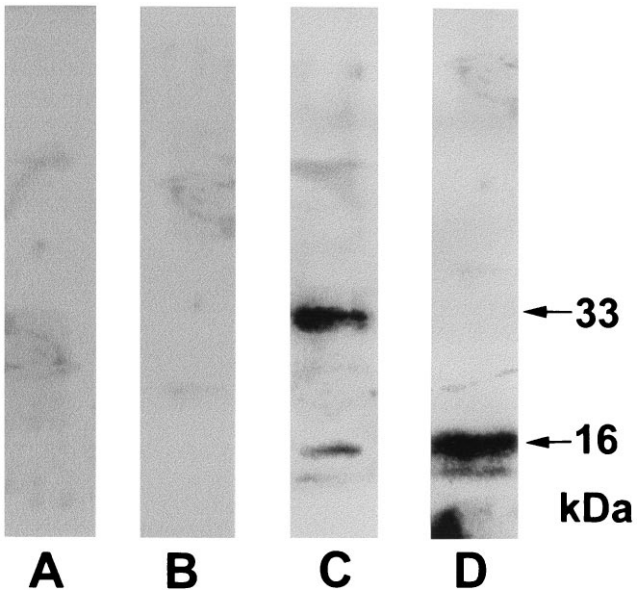

FIG. 4. Western blot analysis of cytosolic and nuclear protein extracts from control and IL-1α33-transfected cells. [Lanes A (cytosolic) and B (nuclear)] Extracts from control MC. [Lanes C (cytosolic) and D (nuclear)] Extracts from IL-1α33 transfectants. Arrows denote the 33- and 16-kDa IL-1α segments.

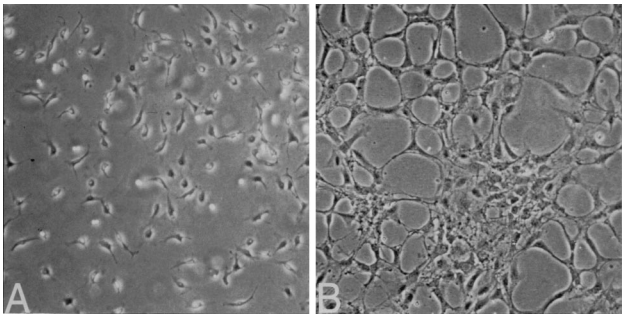

FIG. 5. Growth patterns of transfected MC. Cells transfected with vector alone display the typical MC pattern of growth as nonorganized, shingle-shaped cells (*A*), whereas 16-kDa N-terminal IL-1α transfectants are elongated and refractile and grow in an organized reticular pattern with a second, larger cell type localized to the interstices (*B*). (×100.)

morphologic phenotypes. These morphologic alterations were independent of specific plasmid integration sites, as Southern analysis of clonal genomic DNA revealed distinctive restriction digest patterns, with a range of one to three integrated cDNA copies per cell (not shown). Cells transfected with the entire IL-1α33 cDNA generally resembled the controls, although several clonal populations that had high levels of the N-terminal propiece nuclear staining also developed the organized reticular network (not shown).

While control transfected MC were not capable of anchorage-independent growth in soft agar, multiple clones expressing the IL-1α N-terminal propiece rapidly formed colonies with high efficiency (23–42%) in soft agar, achieving sizes of >100 cells per colony (Fig. 6 *A–C*). Cells expressing NLS-mutagenized NLS did not exhibit altered culture morphology and were incapable of growth in soft agar (Fig. 6*D*).

The failure of the NLS-mutated IL-1α constructs to concentrate within the nucleus or to transform cells is consistent with a requirement for active, rather than diffusional nuclear transport. However, the 16-kDa size of the N-terminal propiece is just below the nuclear envelope diffusional limit, which occurs between 17 and 40 kDa (27). To rigorously demonstrate that the nuclear localization, and the subsequent cell transforming activity of the IL-1α N-terminal propiece, is the consequence of active transport, a 54-kDa N-terminal IL-1α/*Drosophila* ADH fusion protein was expressed in MC cells. Cells expressing the ADH protein alone demonstrated exclusively cytoplasmic staining (Fig. 7*A*), whereas the IL-1α/ADH fusion protein was concentrated in the nucleus, as determined by ADH histochemical (Fig. 7*B*) and N-terminal IL-1α immunofluorescence staining (Fig. 7*C*). The fusion protein retained the ability to transform and yielded large colonies in the soft agar assay (Fig. 7*D*).

To further assess the extent of neoplastic transformation, three individual clones (clones 3.3, 4.5, and 5.7) expressing high levels of the IL-1α N-terminal propiece were introduced into the peritoneal cavities of nu/nu mice. Control MC transfected with the expression vector alone did not yield tumor formation ($n = 6$), whereas mice injected with the N-terminal transfectants developed massive tumor formation with each studied clone (clone 3.3, 5/6 mice; clone 4.5, 6/6 mice; clone 5.7, 6/6 mice). The histology of the tumors closely resembled the *in vitro* growth patterns, with highly organized networks of elongated or spindle-shaped cells, and with larger, rounded cells in the interstices (Fig. 8*A*). The tumors were highly invasive (Fig. 8*B*) and demonstrated exuberant neovascularization (Fig. 8*C*). Culture of recovered tumor cells yielded G418-resistant colonies with the same morphologic phenotype and retained expression of the IL-1α N-terminal fragment (not shown).

DISCUSSION

These data indicate that the primary IL-1α translation product undergoes a unique pattern of processing, giving rise to two proteins with distinctive functional characteristics: (*i*) the 17-kDa C-terminal segment, which acts as a classical cytokine

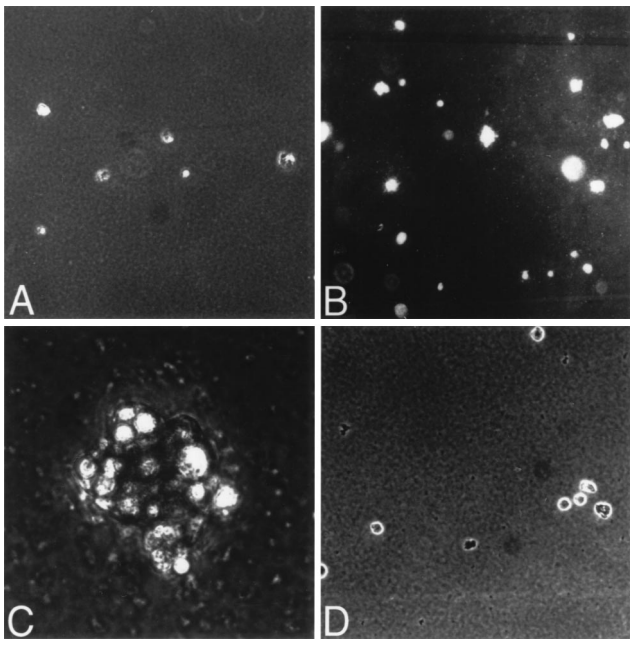

FIG. 6. Soft agar transformation assay of transfected MC. Control MC, transfected with vector alone, did not grow in suspension (*A*), whereas cells expressing the 16-kDa N-terminal IL-1α propiece form multicellular colonies with high efficiency (*B* and *C*). Cells expressing the NLS-mutated N-terminal IL-1α propiece did not grow in suspension (*D*). (*A–D*; ×75.)

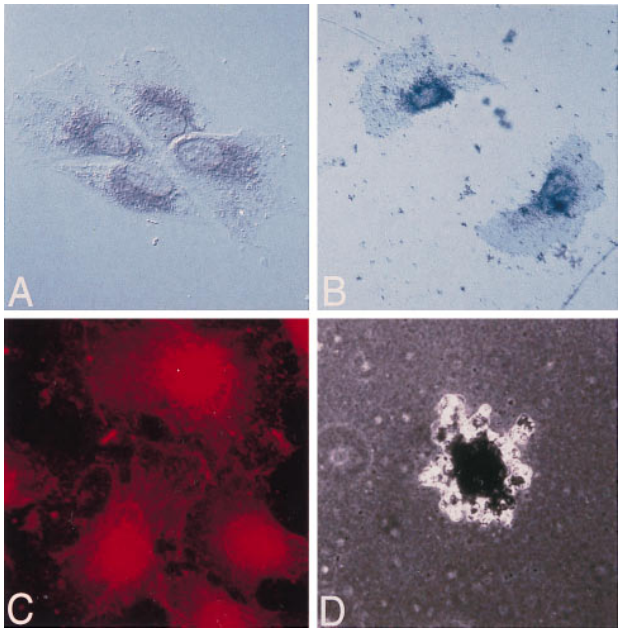

FIG. 7. Nuclear targeting of N-terminal IL-1α propiece/*Drosophila* ADH fusion protein. Cells transfected with ADH cDNA alone demonstrated exclusively cytosolic staining (*A*), whereas both components of the IL-1α propiece/ADH fusion protein were concentrated in the nucleus as demonstrated by ADH staining (*B*) and immunofluorescence staining for the propiece (*C*). Cells expressing IL-1α propiece/ADH fusion protein developed large colonies in agar suspension (*D*). (*A–C*, ×750; *D*, ×85.)

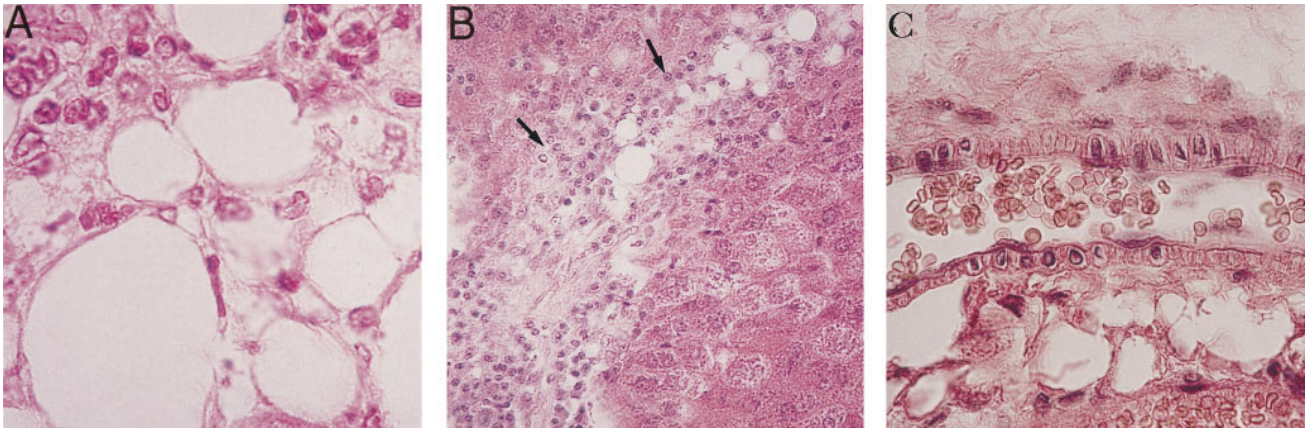

FIG. 8. Histology of tumors induced by 16-kDa N-terminal IL-1α propiece transfected cells (hematoxylin/eosin stain). (*A*) The tumors display the same growth patterns observed *in vitro*, consisting of elongated, spindle-like cells, with a second epithelioid cell in the interstices. (*B*) The tumor were highly invasive, with extensive infiltration of all visceral organs, including the liver shown here (arrows), and demonstrated exuberant neovascularization with palisading endothelial cells (*C*). (*A* and *C*, ×500; *B*, ×330.)

by binding to IL-1 plasma membrane receptors and (*ii*) the 16-kDa N-terminal IL-1α propiece, which is actively transported to the nucleus, where it presumably has a role under normal conditions in the regulation of cell growth or differentiation. The functionally bipartite nature of the IL-1α precursor is unique and distinguishes it from other NLS-containing growth factors, such as basic fibroblast growth factor and platelet-derived growth factor, which, in spite of the presence of NLSs, have an absolute requirement for the exogenous (i.e., receptor-mediated) pathway for their transforming properties (28–30). Analysis of the sequenced forms of IL-1α indicates an absolute conservation of the NLS in all known mammalian sequences, including sheep, cow, rabbit, rat, and mouse. The NLS does not appear to be functional when expressed in the context of the intact 33-kDa precursor molecule but does function when expressed as a fusion protein with *Drosophila* ADH. These findings suggest that the activity of the NLS is latent in the intact precursor and that proteolytic processing results in the development of nuclear localizing activity, presumably due to a conformational change. The finding that a limited peptide sequence containing the IL-1α NLS is completely sufficient to mediate active nuclear transport of heterologous proteins indicates that additional, non-NLS regions of the molecule are not required for the establishment of N-terminal IL-1α nuclear transport.

It should be noted that others have reported on the nuclear translocation of the 17-kDa C-terminal form of IL-1α when complexed to the plasma membrane receptor (13, 14). This translocation process is mediated by a NLS present in the receptor protein and was demonstrated using ^{125}I-labeled C-terminal IL-1α in cells with relatively high levels of plasma membrane receptors (thymoma EL 4 and diploid fibroblasts; ref. 31). Given the fact that the cells evaluated in our study express very low levels of IL-1 receptors (31), we were not able to detect C-terminal IL-1α/receptor complexes in the nucleus using our methods of analysis.

These observations extend the work of Wessendorf *et al.*, who found that mutation of K_{82} within this region eliminated precursor IL-1α nuclear targeting in transfected NIH 3T3 cells (32). Since the NIH 3T3 cells transfected in that study neither synthesize IL-1α nor process the precursor into the N- and C-terminal segments, the differential patterns of IL-1 distribution reported in our studies were not seen. This differential distribution, with resultant high nuclear concentrations of the N-terminal propiece alone, may be a requirement for transformation, as 31-kDa IL-1α-transfected NIH 3T3 cells did not evidence any significant morphologic or functional changes (32). The specific nuclear targeting of the N-terminal IL-1α propiece may be regulated by several posttranslational events. The propiece includes residues $K_{82}K_{83}$, which undergo specific N-ε amino myristyl acylation within human monocyte lysates (17), and it is conceivable that acylation, with resultant loss of positive charge, regulates the activity of the NLS.

Given the multistep nature of malignant transformation, it is unlikely that the 16-kDa N-terminal IL-1α propiece is a complete oncoprotein, so cooperation with other oncoproteins or inactivation of tumor suppressor genes is likely. Additionally, transformation may be cell context-dependent. The MC that were transformed in this report acquired a remarkably broad, pluripotential pattern of behavior in culture (33, 34). In contrast, overexpression of the IL-1α propiece in rat-1 fibroblasts does not lead to malignant transformation (unpublished observations), whereas overexpression of the 31-kDa IL-1α precursor in a previously transformed endothelial cell line not capable of endogenous IL-1α synthesis or processing was actually associated with diminished growth rates (35). The highly invasive and vascularized nature of the IL-1α-mediated tumors presumably reflects the fact that these cells synthesize large quantities of angiogenic basic fibroblast growth factor and transforming growth factor type β, as well as the tissue invasion facilitator gelatinase A (33, 34, 36, 37). While the MC-derived spindle cell tumors do not fully manifest the histopathologic features of Kaposi sarcoma, the cytokine secretory profiles (including IL-1α) and vascular cell origins of these tumors exhibit remarkable similarity and suggest that nuclear IL-1α may play a critical role in the generation of the Kaposi sarcoma spindle cell phenotype.

The generation of a potentially oncogenic protein by post-translational proteolytic cleavage implies that control of the processing step or sequestration by an intracellular receptor antagonist could play a critical role in regulating the extent of the nuclear targeting of the N-terminal IL-1α propiece. In this regard, several 31-kDa IL-1α transfectants appeared to have augmented levels of proteolytic processing, with higher nuclear concentrations of the propiece and increased transforming ability as assessed by soft agar growth and formation of tumors in mice. The IL-1α processing enzyme calpain is translated as a proenzyme that undergoes autoproteolytic cleavage; its activity is then regulated by phosphorylation and by a specific inhibitor, calpastatin (38). These events may be important in control of IL-1α processing and intracellular distribution. IL-1α has been identified as an autocrine growth factor in several forms of human myelogenous leukemia and is expressed at high levels by human T cells infected with the retrovirus human T-lymphotropic virus type 1 (7, 39). Intriguingly, these cells are also characterized by augmented expres-

sion of calpain (40), suggesting that a causal link may exist between increased IL-1α synthesis, calpain-dependent processing, and malignant transformation resulting from enhanced nuclear concentrations of the IL-1α N-terminal propiece.

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