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Consensus-Interferon and Platelet-Derived Growth Factor Adversely Regulate Proliferation and Migration of Kaposi's Sarcoma Cells by Control of c-*myc* Expression

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Platelet-derived growth factor-B (PDGF-B) is a potent paracrine-acting mitogen in Kaposi's sarcoma (KS) lesions. Interferon-α is widely used for clinical treatment of KS. Here we show that platelet-derived growth factor-B activates proliferation and migration of cultivated AIDS-KS spindle cells whereas interferon-α acts as an inhibitor. At the molecular level, these opposite activities of platelet-derived growth factor-B and interferon-α converged onto the adverse regulation of the c-myc gene expression. Platelet-derived growth factor-B induced c-myc mRNA and protein synthesis in cultivated AIDS-KS spindle cells whereas interferon-α inhibited these processes. Using c-myc-specific phosphothioate antisense oligodeoxynucleotides, we demonstrated that down-regulation of c-myc expression is sufficient to inhibit proliferation and migration of KS spindle cells in vitro. This indicated that c-Myc protein may be an important regulatory molecule of KS spindle cell proliferation and migration. High amounts of the c-Myc protein were detected in the nuclei of KS spindle cells in histological sections of AIDS-KS biopsies. This suggested that the c-myc gene may also regulate proliferation and migration of AIDS-KS spindle cells in vivo. In this case, c-myc may play an important role in the focus of major pathogenic and therapeutic pathways of KS. (Am J Pathol 1996, 149:1871–1885)

Kaposi's sarcoma (KS) is a frequently occurring tumor in patients with the acquired immune deficiency syndrome (AIDS).[1,2] Characteristic for early KS lesions are networks of thin-walled, dilated vessels with irregular outlines, lined by flattened endothelium (for review see Refs. 3 and 4). In addition, prominent clusters of spindle-shaped cells are evident. These so-called spindle cells are considered to be the tumor cells of the lesions.[3,4]

As the major processes and factors involved in the initiation of AIDS-KS development, deregulation of the cytokine network in the serum of human immunodeficiency virus (HIV)-infected patients, the HIV Tat protein, and a novel human herpesvirus called HHV-8 have been discussed.[3–7] Although it is unknown which of the above factors may initiate KS development, it is commonly accepted that, subsequent to initiation, KS progression is regulated by a reactive interaction of several different cell types mediated by paracrine action of cytokines and growth factors.[3–5]

Recently we have shown that cultivated AIDS-KS spindle cells exhibit a pattern of gene expression identical to that of KS spindle cells *in vivo* with respect to numerous cytokines (platelet-derived

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growth factor (PDGF)-B, interleukin (IL)-1β, IL-1α, IL-6, and oncostatin M) and their receptors.[8,9] In addition, the effects of these cytokines and growth factors on the AIDS-KS spindle cell cultures *in vitro* were reflected by the gene expression observed in KS lesions *in vivo*.[9]

PDGF-B, a major serum mitogen, was found to be a potent paracrine-acting mitogen for cultivated AIDS-KS spindle cells *in vitro*.[10,11] High expression of PDGF β-receptor in these cells and a lack of PDGF-B synthesis supported this observation at the molecular level.[11] In KS lesions *in vivo*, PDGF-B was synthesized from subpopulations of cells that were intermingled with the spindle cells.[8,9] The KS spindle cells themselves were negative for PDGF-B expression but highly positive for PDGF β-receptor mRNA and protein.[8,9] These data suggested that PDGF-B may also activate proliferation of KS spindle cells *in vivo* by paracrine mechanisms.

Interferon (IFN)-α is among the most commonly used agents for medical treatment of KS.[12] It causes significant regression of KS lesions in approximately 40% of the patients.[13] It has been suggested that it is the indirect effects of IFN-α, such as its immunomodulatory activity,[14] or its potential capability to inhibit KS angiogenesis by down-regulation of basic fibroblast growth factor (bFGF) expression in KS cells,[15] that are related to the therapeutic efficacy of IFN-α. Very little is known, however, whether and through which mechanisms IFN-α may act directly on KS spindle cells in medical treatment of KS.

Besides PDGF and IFN-α, a wide variety of growth factors and cytokines are involved in the pathogenesis and therapy of KS. It is tempting to speculate that the different activating and inhibitory factors may focus on central regulatory molecules that trigger proliferation and migration of the KS spindle cells. In particular, the product of the proto-oncogene c-*myc* may be such a central regulatory molecule. It has been shown that PDGF, a major KS pathogen, and IFN-α, a major KS inhibitory factor, are both able to modulate c-*myc* gene expression in certain cell types.[16–20]

In this study we show that c-*myc* gene expression as well as proliferation and migration of cultivated AIDS-KS spindle cells are adversely regulated by PDGF-B and IFN-α. Specific inhibition of c-*myc* expression with antisense oligodeoxynucleotide (ODN) technology revealed that the c-*myc* gene regulates proliferation and migration of cultivated AIDS-KS spindle cells. In addition, we demonstrated a strong expression of the c-*myc* gene in histological sections from AIDS-KS biopsies. This indicated that also *in vivo* c-*myc* may regulate proliferation and migration of KS spindle cells. These data suggest that the c-*myc* gene may be a master switch in the focus of major pathogenic and therapeutic pathways of KS.

Materials and Methods

Growth Factors and Cytokines

Recombinant PDGF-B and bFGF were purchased from Boehringer Mannheim (Mannheim, Germany). Recombinant IFN-α (specific activity, 1×10^9 U/mg protein) designated IFN-Con1 was generously provided by Amgen.

Oligodeoxynucleotides

Phosphothioate-modified ODNs purified by high pressure liquid chromatography were purchased from Biognostik and from Birsner and Grob Biotech (Denzlingen, Germany). The sequence of the c-*myc*-specific antisense oligonucleotide (asODN; 5′-AAC GTT GAG GGG CAT-3′) was complementary to the translation initiation site of c-*myc* mRNA. A sense oligonucleotide (sODN; 5′-ATG CCC CTC AAC GTT-3′) with a secondary structure identical to that of the asODN, and a scrambled oligonucleotide (scrODN; 5′-CTG AAG TGG CAT GAG-3′) with no homology to any known human gene and with the same G/C content as the asODN served as controls. The asODN used in this study had been successfully used previously by numerous other laboratories for specific inhibition of c-*myc* expression.[21–25]

AIDS-KS Spindle Cell Cultures

AIDS-KS spindle cell cultures were established by explant culture method from KS biopsies of the skin of six male AIDS patients. KS cultures were maintained in Dulbecco's minimal essential medium (DMEM)/10% fetal calf serum (FCS) and characterized as described previously.[9,26] The following AIDS-KS cultures were used in these studies: M7/3, M5/1, M8/2, M9/1, M3/4, and H1/3. The experiments described in this paper were carried out with cultures with a passage number between three and seven.

Measurement of DNA Synthesis and Proliferation

The mitogenic activity of PDGF-B and IFN-Con1 on AIDS-KS spindle cell growth was determined as the ability to stimulate incorporation of [^{3}H]thymidine into DNA of cultivated AIDS-KS spindle cells. Cells were seeded into 24-well plates (Costar Europe Badhoeve-

dorp, The Netherlands) at a density of 5×10^4 cells/well and allowed to adhere for 24 hours in DMEM/10% FCS. Subsequently, cells were incubated in DMEM/0.5% FCS for 48 hours and, after the addition of the respective factors in DMEM/0.5% FCS, for an additional 20 hours. After this incubation, [methyl-^{3}H]thymidine (Amersham, Little Chalfont, UK) was added to a final concentration of 1 μCi/ml for 4 hours. Finally, cells were washed, trypsinized, and harvested with a cell harvester, and incorporated radioactivity was determined by liquid scintillation counting.

In addition, proliferation experiments were carried out. AIDS-KS spindle cells were seeded into 24-well plates at a density of 1×10^4 cells/well. Cells were incubated for 9 days in DMEM/0.5% FCS containing the respective factors, with changes of medium and supplements every 3 days. After this incubation period, cells were trypsinized and cell number was determined with a Coulter counter (Coulter Electronics, Hialeah, FL).

Migration Assay

Migratory activity of AIDS-KS spindle cells was assayed in a modified Boyden chamber.[27] The lower compartment of the Boyden chamber was filled with a solution of the respective factor (PDGF-B, IFN-Con1, and ODN) in DMEM and the upper compartment was filled with 600 μl of cell suspension (1.8×10^5 cells/ml). Each assay was set up in triplicate. The chambers were incubated at 37°C for 4 hours. Filters were removed from the chambers, and the cells on the filters were fixed in methanol and stained with eosin G and thiazine dye. Cells on the upper surface of the filters were wiped off and the filters were mounted on a microscope slide. The numbers of migrated cells were determined in 42 different microscopic fields of each filter (magnification, ×160) with a computer-assisted analysis system (Optimas image analysis system, Stemmer, Puchheim, Germany). The means ± SD of all 42 fields of each triplicate assay were calculated and are given in the results.

Synthesis of Hybridization Probes

The ^{32}P-labeled DNA hybridization probes were prepared using a random primer oligonucleotide kit (Boehringer Mannheim). The c-*myc* probe was from a 1500-bp *Sst*I fragment coding for c-*myc* exon 2 (Amersham Buchler, Braunschweig, Germany).

Northern Blot Analysis

AIDS-KS spindle cells were seeded subconfluently in DMEM/10% FCS overnight, after which medium was replaced with DMEM/0.5% FCS for 48 hours. Subsequently, PDGF-B or PDGF-B and IFN-Con1 were added for 4 hours. Total cellular RNA was isolated by the acidic phenol method as described,[28] and 20 μg of the RNA was size fractionated on a vertical 1% agarose/6% formaldehyde gel in the presence of 1 μg/ml ethidium bromide. The RNA was transferred to GeneScreen plus nylon membrane (NEN, Boston, MA) by a vacuum blotting device (Millipore, Bedford, MA) and covalently linked by 5 minutes of ultraviolet irradiation. Hybridizations were performed at 42°C for 16 hours in 50% formamide, 5X standard saline citrate (SSC), 10% dextran sulfate, 1% Sarkosyl NL-30, 100 μg/ml sonicated salmon sperm DNA, and 250 μg/ml tRNA. A total of 2×10^6 cpm/ml of radioactively labeled probe (sp. act. $\geq 5 \times 10^8$ cpm/μg DNA) was used for hybridization. After hybridization, filters were washed with a final stringency of 0.1X SSC, 0.1% Sarkosyl NL-30 at 50°C. Autoradiography was performed with an intensifying screen at −70°C.

Immunofluorescence

AIDS-KS spindle cells were seeded in eight-well chamber slides in DMEM/10% FCS. After adherence of cells, medium was replaced with DMEM/0.5% FCS for 48 hours. Subsequently, the respective factors (PDGF-B, bFGF, asODN, and scrODN) were added in DMEM/0.5% FCS for the respective periods of time (c-*myc* induction, 4 and 16 hours; c-*fos* induction, 1 hour). To allow uptake of ODN in the shorter period of time required for c-*fos* induction, ODNs were applied to the cells 3 hours in advance and again simultaneously with addition of PDGF-B. Subsequently, cells were fixed in methanol at −20°C for 15 minutes and subjected to immunohistochemical procedures. Nonspecific binding was blocked by incubation with normal sheep serum (Dianova, Hamburg, Germany) for 30 minutes at 37°C. Samples were incubated with a monoclonal anti-c-Myc antibody (Oncogene Science, Hamburg, Germany; diluted 1:20 in phosphate-buffered saline (PBS))[29] or a monoclonal anti-c-Fos antibody (Oncogene Science; diluted 1:20 in PBS) for 2 hours at room temperature. To detect anti-c-Myc antibody, either a Cy3-coupled anti-mouse secondary antibody (Dianova; 1:2000) or a 5(6)-carboxy-rhodamine 101-*N*-hydroxysuccinimide ester (RHODOS)-coupled anti-mouse secondary antibody (Boehringer Mannheim; 1:10) was used. A fluorescein isothiocyanate (FITC)-coupled anti-mouse secondary antibody (Boehringer Mannheim; 1:50) was used to detect anti-c-Fos antibody. In every case, incubation was carried out for 1 hour.

Washing steps were performed between the different incubations (three times for 5 minutes each with PBS). Staining was evaluated on a fluorescence microscope (Zeiss, Oberkochen, Germany).

Western Blot Analysis

AIDS-KS cells were plated subconfluently (3×10^6 cells/dish) in six-well dishes (Costar Europe) in DMEM/FCS 10% for 24 hours. The Medium was then replaced with DMEM/0.5% FCS, and after additional incubation for 48 hours, medium was supplemented with the respective factors (PDGF-B, IFN-Con1, or ODN). At 4 and 16 hours later, cells were lysed in 100 μl of 2X polyacrylamide gel electrophoresis buffer (130 mmol/L Tris/HCl, pH 6.8, 10% sodium dodecyl sulfate (SDS), 20% glycerol, 1.4 mol/L β-mercaptoethanol, 0.01% bromphenol blue), harvested with a rubber policeman, and snap-frozen in liquid nitrogen. Cell extracts (80 μl) were loaded on a 12.5% SDS-polyacrylamide gel. After electrophoresis, proteins were transferred to nitrocellulose (0.22 μm pore size; Schleicher and Schüll, Dassel, Germany). Nonspecific binding sites were blocked by incubation for 1 hour in TBST (10 mmol/L Tris, pH 8.0, 150 mmol/L NaCl, and 0.05% Tween 20) supplemented with 5% nonfat milk powder. c-Myc protein was detected by incubating the blot first with a mouse monoclonal anti-c-Myc antibody (1:10 in TBST)[30] for 10 hours at 4°C, and after washing in TBST (three times for 5 minutes each) with alkaline-phosphatase-conjugated goat anti-mouse antibody (Serva, Heidelberg, Germany) for 60 minutes at room temperature (1:7500 in TBST). After washing away excess antibody, the filter was developed using the BCIP/NBT staining system (Promega Corp., Madison, WI). Band intensity was quantified using a Vilbert Lourmat BIOID V 6.02c system.

Uptake of Deoxyglucose

Uptake of 2-[^{3}H]deoxyglucose was essentially measured as described previously.[19] Cells were seeded into 24-well plates and cultured in DMEM/10% FCS. After 24 hours, medium was replaced with DMEM/0.5% FCS, and 48 hours later, cells were preincubated for 30 minutes in glucose-free DMEM supplemented with PDGF-B (2 nmol/L) and the respective ODN. Subsequently, 2.5 μCi of 2-[^{3}H]deoxyglucose (0.2 mmol/L; Amersham) was added. After 15 minutes of incubation, cells were washed and trypsinized, and cell-bound activity was measured by liquid scintillation counting.

Cytotoxicity Assay

A total of 1×10^4 AIDS-KS spindle cells was cultured in 24-well plates in DMEM/0.5% FCS. Cytotoxicity of IFN-Con1 (in concentrations ranging between 0.08 ng/ml and 80 ng/ml) or as/s/scrODN (3, 6, and 10 μmol/L) was determined by trypan blue dye (0.5% in 0.85% saline) exclusion at days 1, 3, and 5.

Immunohistochemistry

Biopsy specimens for immunohistochemical detection of c-Myc protein were obtained from nine HIV-infected patients in Centers for Disease Control (CDC) group C.[31] All patients belonged to the risk group of homosexuals and were treated with 3′-azido-3′-deoxythymidine (AZT). All biopsies were removed for diagnostic purposes with the informed consent of the patients. KS development was still in progress in all patients when KS biopsy specimens were obtained. No KS-specific medical treatment was applied.

Immediately after removal, the biopsy tissues were fixed in 4% paraformaldehyde at 4°C overnight. Dehydration and embedding in paraffin was carried out as described previously.[8,9] Tissue sections were incubated with a monoclonal anti-c-Myc antibody (Oncogene Science; dilution, 1:20 in PBS)[29] for 1 hour at room temperature. Bound primary antibody was detected by using a biotin/streptavidin-based detection kit (Super Sensitive Detection Kit, BioGenex, San Ramon, CA) according to the supplier's instructions. Staining was performed with fast red chromogen for 30 minutes. After completion of the staining reaction, sections were counterstained in Mayer's hematoxylin. In additional control experiments, the primary antibody was replaced with bovine serum albumin. No staining was observed in these experiments. The Super Sensitive Detection Kit was also used together with heterologous antibodies for the detection of several different antigens (von Willebrand factor, PDGF β-receptor, monocyte chemoattractant protein-1) in KS tissue sections obtained from the same paraffin blocks as used in this study. Clearly different patterns of staining were obtained in these studies; in no case was nonspecific background staining observed (data not shown).

Results

c-myc *Expression in Cultivated AIDS-KS Spindle Cells Is Adversely Regulated by PDGF-B and by IFN-Con1*

Quiescent AIDS-KS spindle cells did not synthesize significant amounts of c-*myc* mRNA, as measured by

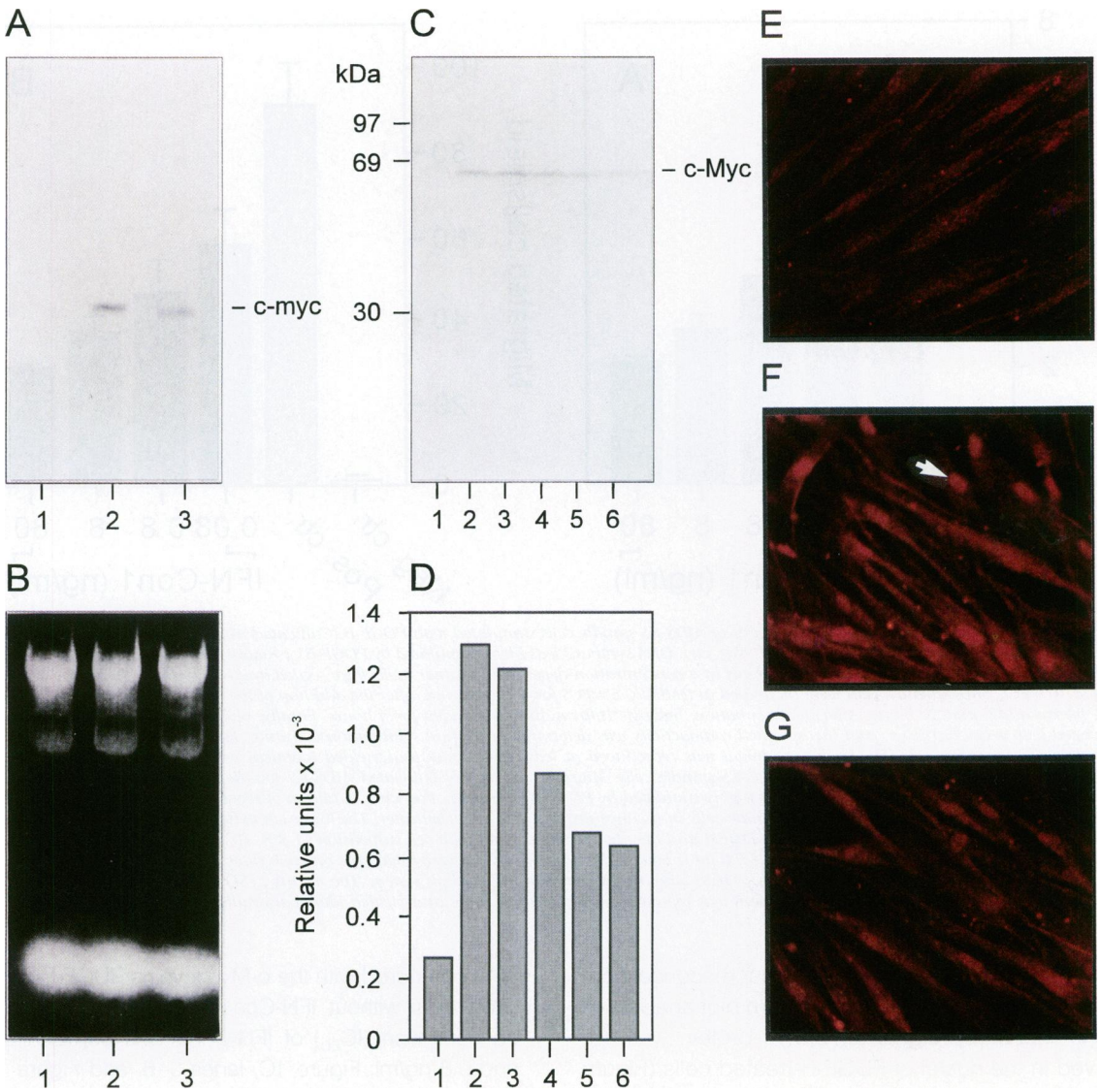


Figure 1. A: *Northern blot analysis for* c-myc *expression in AIDS-KS spindle cells. Cells were incubated for 4 hours in DMEM/0.5% FCS (negative control) or in DMEM/0.5% FCS supplemented with PDGF-B (2 nmol/L) or with PDGF-B (2 nmol/L) plus IFN-Con1 (8 ng/ml). Total RNA was isolated, subjected to electrophoresis, and blotted onto a nylon membrane. Hybridization was carried out with a radioactively labeled human* c-myc *probe (sp. act.* ≥ 5×10^8 *cpm/μg DNA) at 42°C for 6 hours.* **Lane 1,** *negative control (DMEM/0.5% FCS);* **lane 2,** *positive control (PDGF-B, 2 nmol/L);* **lane 3,** *PDGF-B (2 nmol/L) plus IFN-Con1 (8 ng/ml). When incubation with the respective factors was carried out for 4 hours, identical results were obtained. Addition of IFN-Con1 to the medium resulted in a decreased expression of* c-myc. **B:** *Ethidium bromide visualization of blotted RNA indicated that equal amounts of RNA were applied onto each lane.* **Lane 1,** *negative control (DMEM/0.5% FCS);* **lane 2,** *positive control (PDGF-B, 2 nmol/L);* **lane 3,** *PDGF-B (2 nmol/L) plus IFN-Con1 (8 ng/ml).* **C:** *Western blot analysis of c-Myc protein in AIDS-KS spindle cells. AIDS-KS spindle cells were incubated for 4 hours with PDGF-B (2 nmol/L) either alone or in the presence of increasing concentrations of IFN-Con1.* **Lane 1,** *negative control (DMEM/0.5% FCS);* **lane 2,** *positive control (PDGF-B, 2 nmol/L);* **lane 3,** *PDGF-B (2 nmol/L) plus IFN-Con1 (0.08 ng/ml);* **lane 4,** *PDGF-B (2 nmol/L) plus IFN-Con1 (0.8 ng/ml);* **lane 5,** *PDGF-B (2 nmol/L) plus IFN-Con1 (8.0 ng/ml);* **lane 6,** *PDGF-B (2 nmol/L) plus IFN-Con1 (80 ng/ml). IFN-Con1 inhibited the PDGF-B-induced synthesis of c-Myc protein dose dependently.* **D:** *Densitometric evaluation of band intensities from the Western blot shown above* **(C). E** *to* **G:** *Immunofluorescence staining for detection of c-Myc protein in AIDS-KS spindle cells. Incubation with the respective factor was carried out for 16 hours. Photographs were taken applying constant exposure times for all samples (10 seconds).* **E:** *Negative control (DMEM/0.5% FCS) with quiescent cells reveals only low-grade nuclear staining.* **F:** *Positive control (PDGF-B, 2 nmol/L) with rapidly proliferating cells and strong nuclear staining* **(arrow). G:** *Cells stimulated with PDGF-B (2 nmol/L) and incubated with IFN-Con1 (8 ng/ml) display a markedly reduced signal for c-Myc protein.*

Northern blot analysis (Figure 1A, lane 1), and c-Myc protein, as analyzed by Western blot (Figure 1C, lane 1) and immunocytochemistry (Figure 1E). Four hours after incubation with PDGF-B (2 nmol/L, c-*myc* mRNA synthesis was significantly increased in AIDS-KS spindle cells (Figure 1A, lane 2). Western blot analysis demonstrated that the induction of c-*myc* mRNA was associated with an increase of c-Myc protein (Figure 1C, lane 2). Densitometric evaluation of band intensities of the Western blot analysis revealed a 4.6-fold higher amount of c-Myc protein in PDGF-B-stimulated cells compared with quiescent

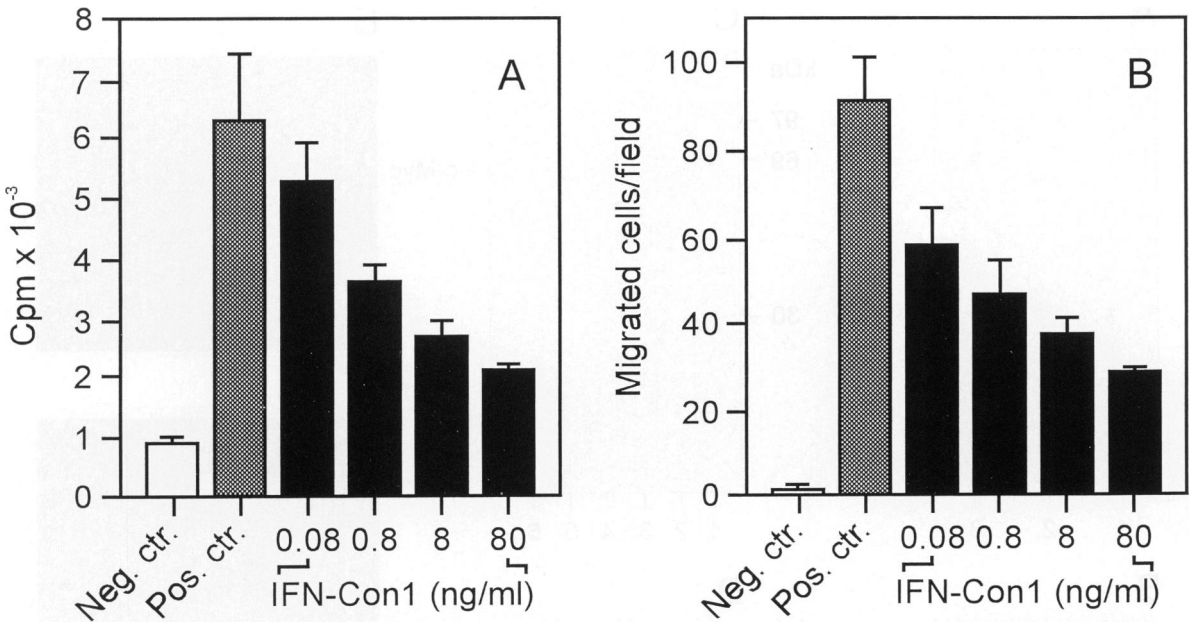


Figure 2. A: *Effects of IFN-Con1 on DNA synthesis of AIDS-KS spindle cells stimulated with PDGF-B. Cultivated AIDS-KS spindle cells showed a low rate of DNA synthesis in the absence of PDGF-B (Neg. ctr). DNA synthesis was highly simulated by PDGF-B (2 nmol/L; Pos. ctr.). Addition of IFN-Con1 to PDGF-B-stimulated cells inhibited DNA synthesis in a concentration-dependent manner. Cells were seeded and allowed to adhere for 24 hours in DMEM/10% FCS. Subsequently, cells were incubated in DMEM/0.5% FCS for 48 hours and, after the addition of the respective factors in DMEM/0.5% FCS, for an additional 20 hours. After this incubation, [methyl-^{3}H]thymidine was added for 4 hours. Finally, cells were washed, trypsinized, and harvested with a cell harvester, and incorporated radioactivity was determined by liquid scintillation counting. Each point represents the mean of a triplicate assay (mean ± SD). Each experiment was reproduced at least three times and yielded identical results.* **B:** *Effects of IFN-Con1 on PDGF-B-induced directional migration of AIDS-KS spindle cells. Migratory activity of cultivated AIDS-KS spindle cells was low in the absence of PDGF-B (Neg. ctr.). Directional migration was highly simulated by PDGF-B (2 nmol/L; Pos. ctr.). Addition of IFN-Con1 to PDGF-B-stimulated cells inhibited directional migration of AIDS-KS spindle cells in a concentration-dependent manner. The lower compartment of a Boyden chamber was filled with a solution of the respective factor in DMEM and the upper compartment with cell suspension (1.8 × 10^5 cells/ml). Each assay was set up in triplicate. The chambers were incubated at 37°C for 4 hours. The numbers of transmigrated cells on each filter were determined in 42 different microscopic fields of each filter (magnification, ×160) with a computer-assisted analysis system. The means ± SD of all 42 fields of each triplicate were calculated and are shown. Each experiment was reproduced at least three times and yielded identical results.*

cells (Figure 1D, bar 2). Immunofluorescence confirmed the data obtained by Western blot analysis. A highly increased signal for c-Myc protein was observed in the nuclei of PDGF-B-treated cells (Figure 1F, arrow) in comparison with unstimulated cells (Figure 1E).

Recently, a novel IFN, IFN-Con1, coding for the most frequent amino acids of eight different leukocyte α-IFNs has been recombinantly produced in *Escherichia coli*.[32] It has been shown that IFN-Con1, in comparison with other available α-IFNs, revealed similar specificity but increased effectiveness against KS cells *in vitro*.[33] For these reasons, IFN-Con1 was used in the following experiments.

IFN-Con1 decreased the PDGF-B-induced synthesis of c-*myc* mRNA (Figure 1A, lane 3) and of c-Myc protein (Figure 1C, lanes 3–6) in cultivated AIDS-KS spindle cells. Densitometric evaluation demonstrated a clearly dose-dependent decrease of c-Myc protein in IFN-Con1-treated cells (Figure 1D, bars 3–6). At the maximal inhibitory concentration of IFN-Con1 (IC_{100} = 80 ng/ml), the level of c-Myc protein was reduced by 66% compared with the c-Myc level in PDGF-B-stimulated cells without IFN-Con1 (half-maximal inhibitory concentration (IC_{50}) of IFN-Con1 was between 0.08 and 0.8 ng/ml; Figure 1C, lanes 3–6, and Figure 1D, bars 3–6). The inhibition of c-Myc protein synthesis by IFN-Con1 was also demonstrated by immunocytochemistry (Figure 1G). Differences between cells treated or not treated with IFN-Con1 were evident, especially with respect to the nuclear staining for the c-Myc protein (Figure 1F, arrow, and Figure 1G).

Proliferation and Migration of Cultivated AIDS-KS Spindle Cells Is Adversely Regulated by PDGF-B and IFN-Con1

Cultivated AIDS-KS spindle cells showed a low proliferation (Figure 2A, Neg. ctr.) and migration rate (Figure 2B, Neg. ctr.) in the absence of PDGF-B. Both proliferation and migration of these cells were highly stimulated by 2 nmol/L PDGF-B (proliferation 6.8-fold (Figure 2A, Pos. ctr.) and migration 46-fold (Figure 2B, Pos. ctr.) compared with unstimulated

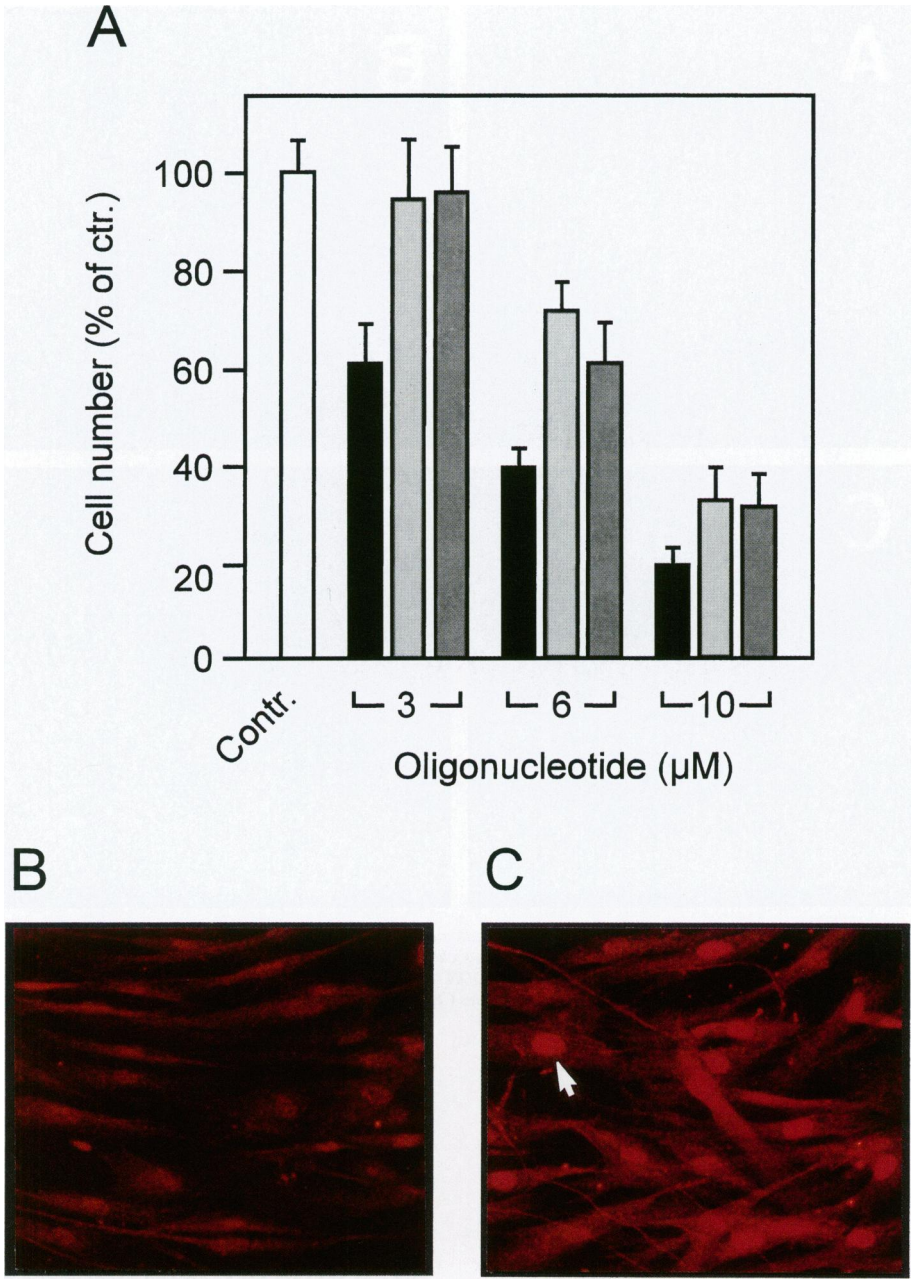


Figure 3. A: *Effects of asODN* (black bars), *scrODN* (bright gray bars), *and sODN* (dark gray bars) *on proliferation of AIDS-KS spindle cells. Cells were cultivated in DMEM/0.5% FCS and stimulated with PDGF-B (2 nmol/L). ODNs were added at day 1 and were replaced at days 3 and 6. Cell number was counted at day 9. Results are expressed as the percentage of cell growth compared with growth of PDGF-B-stimulated control cells in the absence of ODN* (white bar). *PDGF-B-induced cell growth was markedly reduced by addition of asODN directed against c-myc mRNA. Each point represents the mean of a triplicate assay (mean ± SD). Each experiment was reproduced at least three times and yielded identical results.* B *and* C: *Immunofluorescence staining of c-Myc protein in AIDS-KS spindle cells after stimulation with PDGF-B (2 nmol/L) and treatment with ODN. AIDS-KS cells treated with asODN (6 μmol/L;* B*) displayed significantly reduced amounts of c-Myc protein compared with control AIDS-KS cells treated with scrODN* (C). *Photographs were taken applying constant exposure times for all samples (10 seconds).*

control cells), whereas addition of IFN-Con1 inhibited proliferation and migration in a concentration-dependent manner (Figure 2A and Figure 2B).

At maximal inhibitory concentrations of IFN-Con1, values of the DNA synthesis were reduced by 77% (±SD) compared with untreated control cells (0.08 ng/ml ≤ IC_{50} ≤ 0.8 ng/ml). Migration was reduced by 71% (±SD) compared with the control without IFN-Con1 (IC_{50} ≤ 0.08 ng/ml). An additional increase of IFN-Con1 concentration did not result in stronger inhibition in either of the two experiments. No significant toxic effects of IFN-Con1 on cultured

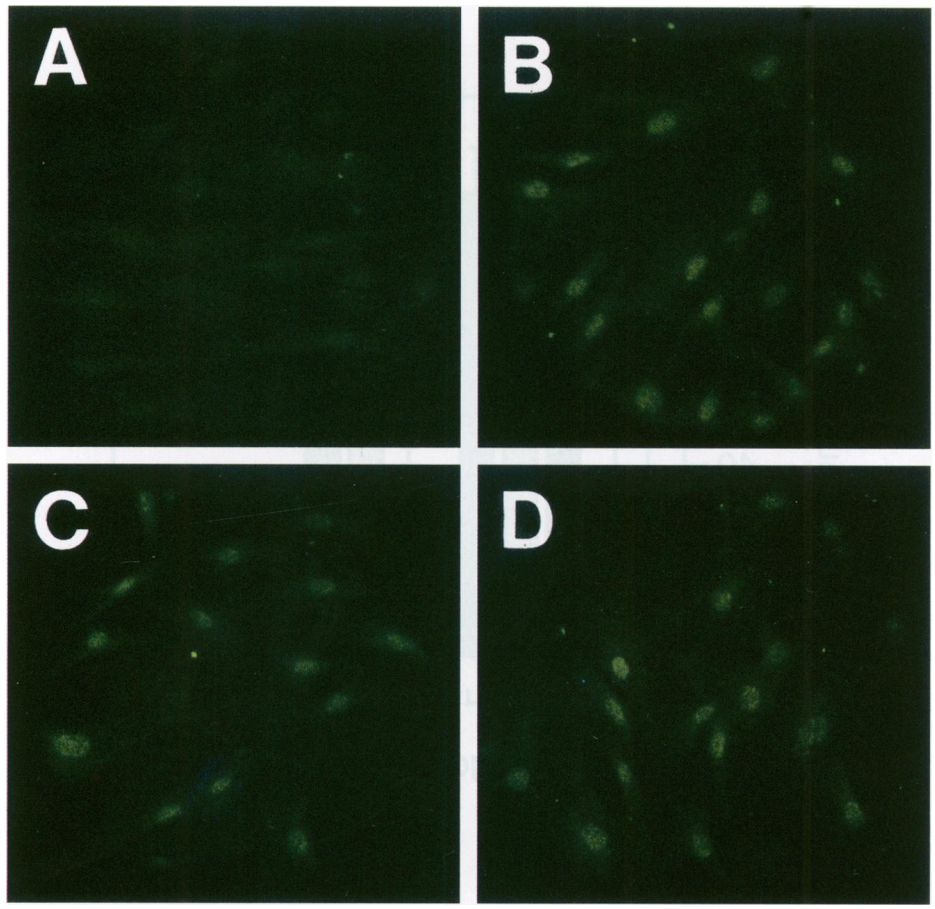


Figure 4. *Immunofluorescence staining of c-Fos protein in AIDS-KS spindle cells after stimulation with PDGF-B (2 nmol/L) and treatment with ODN. In comparison with unstimulated cells (A), synthesis of c-Fos protein was clearly increased in PDGF-B-treated cells (B). In PDGF-B-stimulated cells c-Fos protein was affected neither by the addition of c-myc-specific asODN (C) nor by the addition of scrODN (D) at a concentration of 6 μmol/L. Photographs were taken applying constant exposure times for all samples (10 seconds).*

AIDS-KS spindle cells were observed in a trypan blue dye exclusion assay (data not shown).

c-myc *Regulates Proliferation and Migration of Cultivated AIDS-KS Spindle Cells*

Phosphothioate asODNs directed against c-*myc* mRNA were used for specific inhibition of c-Myc synthesis to investigate whether and to what extent c-*myc* regulates proliferation and migration of cultivated AIDS-KS spindle cells.

Proliferation of PDGF-B-stimulated AIDS-KS cells was clearly inhibited by c-*myc*-specific asODN at a concentration of 3 μmol/L (Figure 3A, black bar, whereas scrODN (bright gray bars), with the same base composition as asODN and sODN (dark gray bars), with structural features identical to those of asODN, exerted no significant inhibitory effects at this concentration (Figure 3A). At higher concentrations (6 and 10 μmol/L), inhibition of proliferation by asODN increased due to non-sequence-specific effects as suggested by the increased inhibitory effects of the control ODN (Figure 3A).

Besides the use of sODN and scrODN, numerous additional controls were performed to prove the specificity of c-*myc* asODN. First, immunocytochemistry revealed that, specifically, asODN decreased c-Myc protein levels (Figure 3B), whereas high levels of c-Myc protein were observed in the nuclei of AIDS-KS spindle cells in the presence of control ODN (Figure 3C, arrow). These data were also confirmed by Western blot analysis (data not shown). Second, the expression of the heterologous c-*fos* gene was not inhibited by c-*myc* asODN in AIDS-KS spindle cells. In comparison with unstimulated cells (Figure 4A), synthesis of c-Fos protein was clearly increased in PDGF-B-treated cells (Figure 4B). In PDGF-B-stimulated cells c-Fos protein was affected neither by the addition of c-*myc*-specific asODN (Figure 4C) nor by the addition of scrODN (Figure

4D). Third, to further demonstrate that the asODN specifically caused inhibition of proliferation by inhibition of c-*myc* expression and not by inhibition of PDGF-B binding to its cognate receptor, we performed 2-[^{3}H]deoxyglucose uptake experiments (Figure 5). The uptake of 2-[^{3}H]deoxyglucose into eukaryotic cells is stimulated by PDGF-B as long as the binding of PDGF-B to its cognate receptor has not been compromised.[19] Indeed, PDGF-B clearly increased the 2-[^{3}H]deoxyglucose uptake into AIDS-KS spindle cells (Figure 5, Pos. ctr.) in comparison with the unstimulated control cells (Figure 5, Neg. ctr.). In increasing concentrations (3, 6, and 10 μmol/L), neither asODN (Figure 5, black bars), scrODN (Figure 5, bright gray bars), nor sODN (Figure 5, dark gray bars) significantly decreased the PDGF-B-mediated 2-[^{3}H]deoxyglucose uptake into AIDS-KS spindle cells. This clearly demonstrated that inhibition of proliferation by asODN was caused by specific inhibition of c-*myc* synthesis rather than by nonspecific effects such as PDGF-B binding to its receptor.

PDGF-B-induced migration of AIDS-KS spindle cells was also inhibited by c-*myc*-specific asODN (Figure 6A). Inhibition at a 1 μmol/L concentration of asODN was 68% compared with the untreated control. At a 3 μmol/L concentration of asODN inhibition increased up to 96%. scrODN as a control caused no significant inhibition of migration of AIDS-KS spindle cells at these concentrations. Filters obtained from the Boyden chamber assay showing migrated cells under various conditions impressively documented this fact (Figure 6, B–I). Migration was highly induced by PDGF-B (Figure 6C) and clearly inhibited by increasing concentrations of asODN (Figure 6, D, F, and H), whereas increasing concentrations of scrODN had only moderate effects on AIDS-KS spindle cell migration (Figure 6, E, G, and I).

Recently, bFGF has been heralded as a major regulator of KS angiogenesis.[6,34] Interestingly, we could show that bFGF (10 ng/ml) induces c-*myc* expression in cultivated AIDS-KS spindle cells (Figure 7B, arrow) in comparison with unstimulated cells (figure 7A) and that inhibition of c-*myc* expression also inhibited bFGF-induced proliferation of AIDS-KS spindle cells (data not shown).

c-myc *Is Highly Expressed in AIDS-KS Spindle Cells* in Vivo

To investigate whether the c-*myc* gene is also expressed in AIDS-KS lesions and thus may also regulate proliferation and migration of AIDS-KS spindle cells *in vivo*, we performed immunohistochemical stainings for c-Myc protein in histological sections from AIDS-KS lesions (Figure 8). With this method, c-Myc protein was predominantly detected in the spindle cells, which are evident by their characteristic longitudinal arrangement in distinct aggregates (Figure 8A, arrow). Spindle cells positive for c-Myc were detected in AIDS-KS lesions of the angioma-like type (Figure 8B, arrow) and also of the fibrosarcomatous type (Figure 8C, arrow). Inflammatory cells present in these KS tissues were negative for c-Myc (Figure 8C, arrowhead). A higher magnification demonstrated nuclear localization of the c-Myc protein (Figure 8D, arrow), which strongly indicates that c-*myc* is active in the regulation of AIDS-KS spindle cell proliferation and migration not only *in vitro* but also *in vivo*. The detection of nuclei of AIDS-KS spindle cells that were negative for c-Myc protein (Figure 8D, arrowhead) proved staining specificity by excluding cross-reactivity of the anti-c-Myc antibody with ubiquitously present non-c-Myc-related nuclear proteins. In an early AIDS-KS lesion, c-Myc protein was also present but in reduced amounts (Figure 8E). As a control for proliferating tissue, the epidermal layer overlaying KS was used. Proliferating basal cells of the epidermis stained clearly positive for c-Myc protein (Figure 8G, arrow). As a negative control, AIDS-KS sections were subjected to the immunohistochemical procedure omitting the primary an-

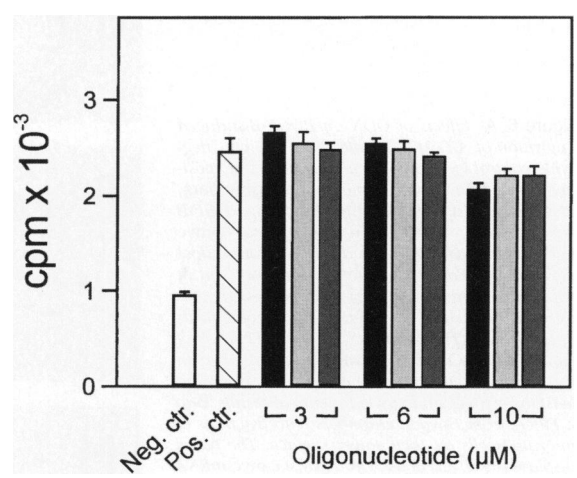


Figure 5. *Effects of asODN* (**black bars**), *scrODN* (**bright gray bars**), *and sODN* (**dark gray bars**) *on 2-[^{3}H]deoxyglucose uptake of AIDS-KS spindle cells. Cells were cultivated for 48 hours in DMEM/0.5% FCS and for 30 minutes in glucose-free DMEM either without any additional additives (Neg. ctr.) or supplemented with PDGF-B (2 nmol/L) either alone (Pos. ctr.) or in combination with increasing concentrations (3, 6, and 10 μmol/L) of the respective ODN. Subsequently, 2.5 μCi of 2-[^{3}H]deoxyglucose was added. After 15 minutes of incubation, cells were washed and trypsinized, and cell-bound activity was measured by liquid scintillation counting. Each point represents the mean of a triplicate assay (mean ± SD).*

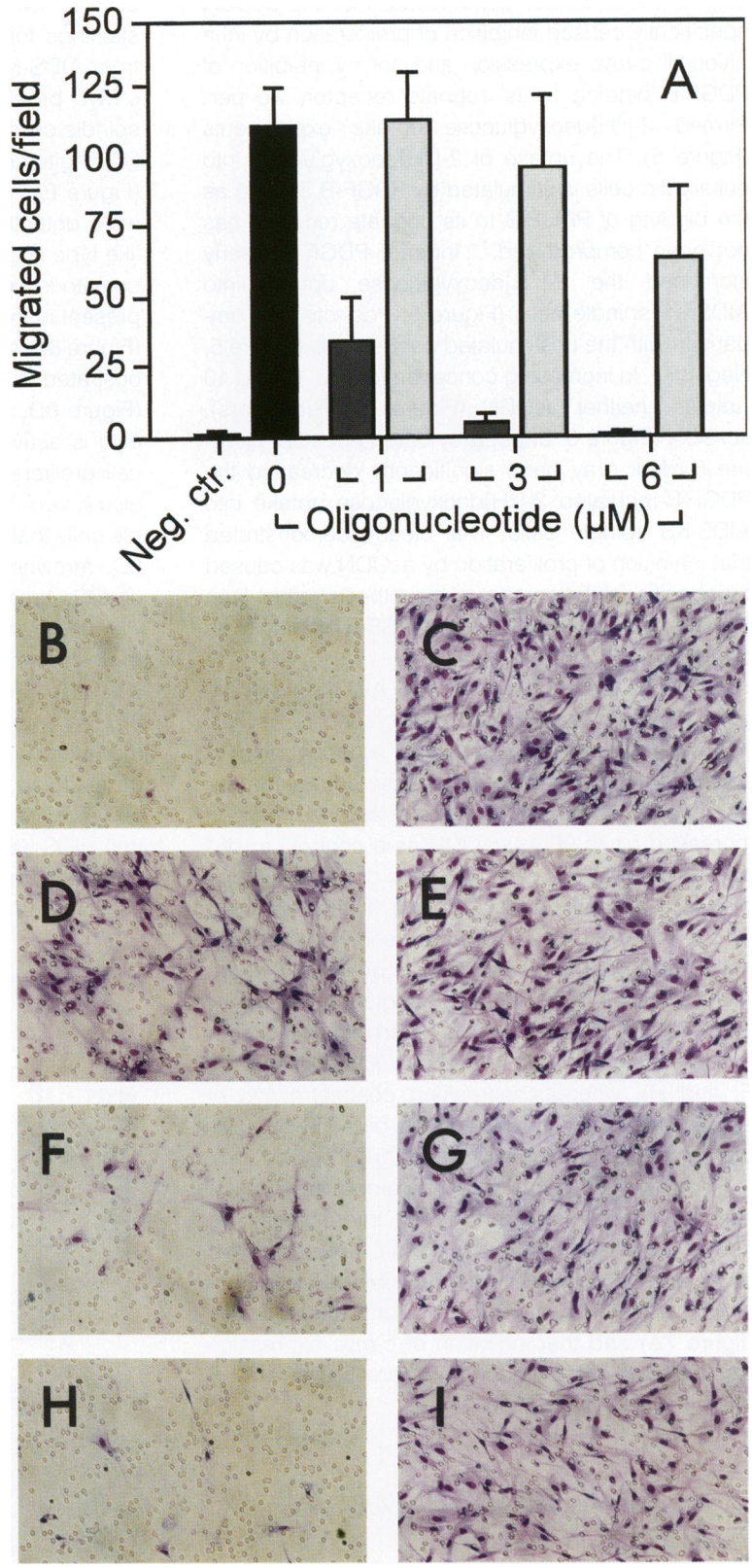


Figure 6. A: *Effects of ODN on PDGF-B-induced migration of AIDS-KS spindle cells. Neg. ctr., negative control (without PDGF-B);* black bar, *positive control (PDGF-B, 2 nmol/L);* dark gray bars, *PDGF-B plus asODN;* bright gray bars, *PDGF-B plus scrODN. asODN directed against c-*myc *mRNA inhibited PDGF-B-induced migration dose dependently. Each experiment was carried out in triplicate. The numbers of transmigrated cells were determined in 42 different microscopic fields of each filter. The means ± SD of all 42 fields of each triplicate were calculated and are given in the results. Each experiment was reproduced at least three times and yielded identical results.* B *to* I: *Filters from Boyden chambers with fractions of migrated cells on their lower surface. The panel displays the effects of asODN against c-*myc *mRNA and of scrODN on PDGF-B-induced migration of AIDS-KS spindle cells.* B: *Negative control (without PDGF-B).* C: *Positive control (PDGF-B, 2 nmol/L).* D, F, *and* H: *PDGF-B (2 nmol/L) plus increasing concentrations of asODN (1, 3, and 6 μmol/L).* E, G, *and* I: *PDGF-B (2 nmol/L) plus increasing concentrations of scrODN (1, 3, and 6 μmol/L).*

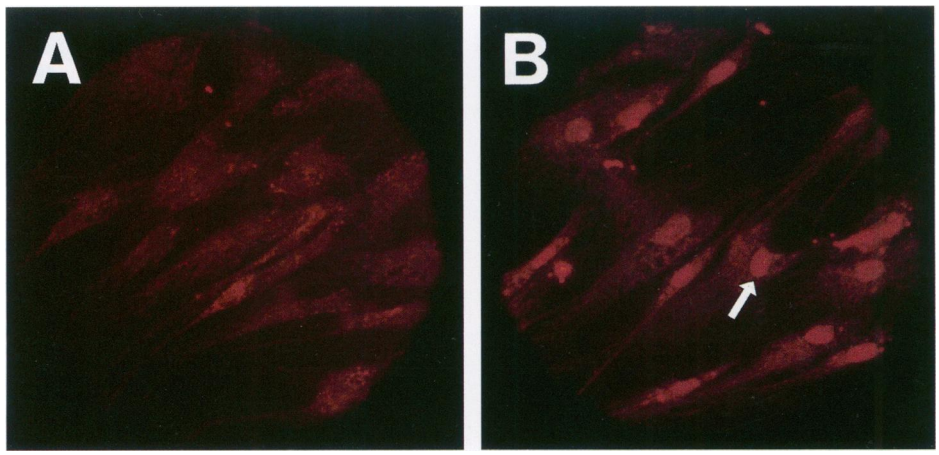


Figure 7. *Immunocytochemical staining of the c-Myc protein in quiescent* (A) *and bFGF-stimulated* (B) *cultivated AIDS-KS spindle cells. Incubation with bFGF was carried out for 4 hours. Photographs were taken applying constant exposure times (10 seconds).*

tibody. In no case was a positive staining observed on these control sections (Figure 8, F and H).

Discussion

In this study we demonstrated that PDGF-B, a major mitogen in AIDS-KS pathogenesis,[8–11] and IFN-Con1, a derivative of IFN-α, which is antitumorigenic in AIDS-KS therapy,[12–15] exercised adverse effects on migration and proliferation of cultivated AIDS-KS spindle cells. The adverse activities of both factors were mediated by the proto-oncogene c-*myc*. This suggested that the activities of different cytokines and growth factors that are active in KS pathogenesis and therapy may converge onto more central regulatory molecules. These regulatory molecules may serve as molecular targets for the development of novel strategies for medical treatment of KS.

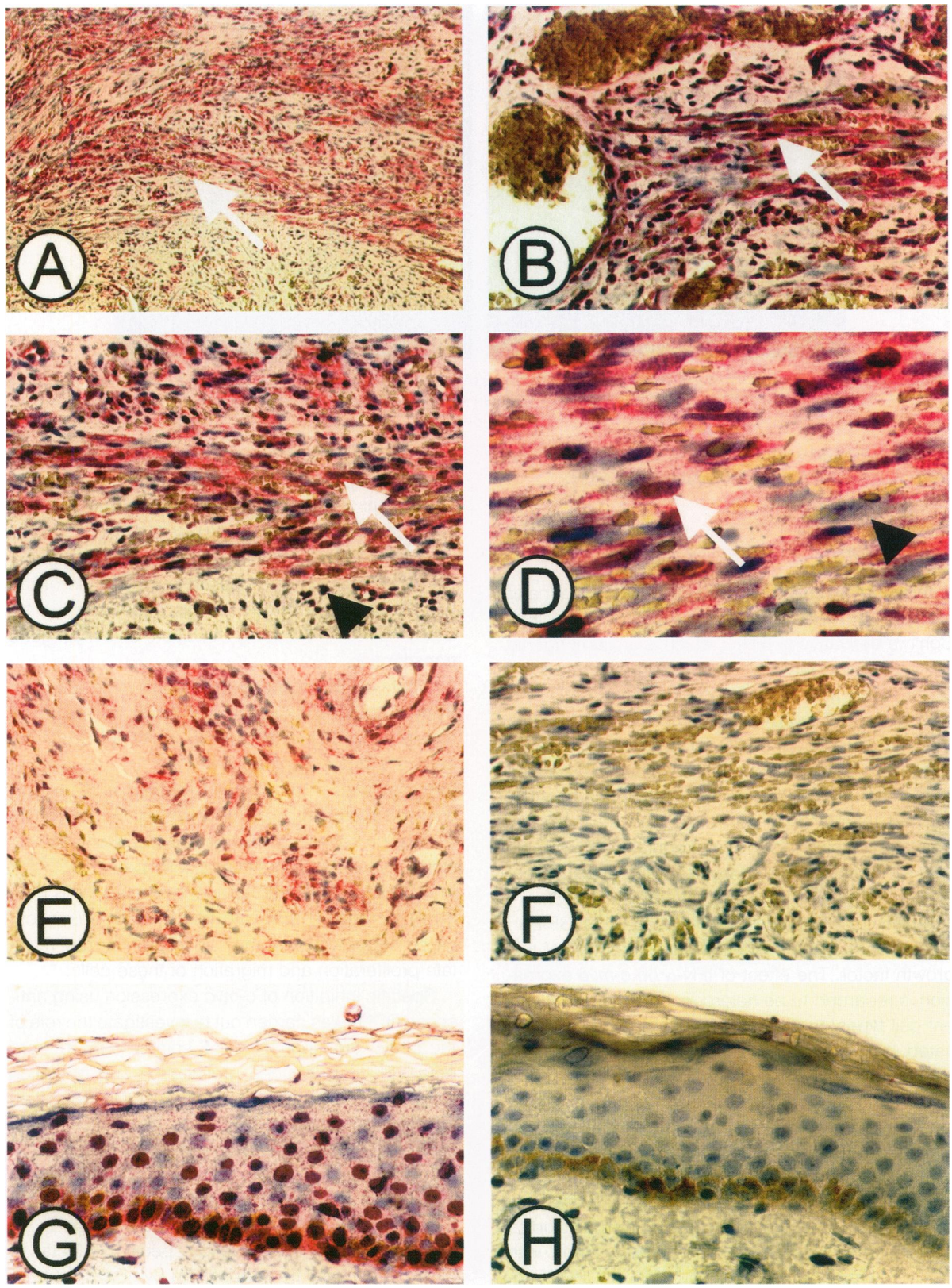
c-*myc* gene expression is induced by PDGF-B in most cells that are susceptible to the action of this growth factor. The effect of IFN-α on c-*myc* expression is regarded to be heterogeneous among different cell types. In certain leukemia cells, IFN-α activated c-*myc* expression,[35] whereas in other cell types, IFN-α had no effect on c-*myc* expression (HL-60 and U937[20]), and inhibition of c-*myc* synthesis by IFN-α has also been reported, for example in lymphoid Daudi cells[20] and renal carcinoma cells.[36] Investigating cultivated AIDS-KS spindle cells, we found that PDGF-induced expression of c-*myc* was markedly down-regulated by IFN-α. Previous studies demonstrated that IFN-α interferes neither with PDGF-B binding to its receptor nor with PDGF-induced receptor activation.[19] In addition, toxic effects of IFN-α as an explanation for the inhibitory effects have not been detected in our experiments. Therefore, we concluded that c-*myc* down-regulation was mediated by IFN-α-specific mechanisms.

Northern blot analysis demonstrated that IFN-α regulated c-*myc* expression in AIDS-KS spindle cells on the RNA level. Down-regulation of c-*myc* expression by IFN-α may be caused by reduction of the half-life of c-*myc* mRNA, as has been reported for Daudi cells,[37] or by the inhibition of c-*myc* gene transcription.

Whatever the responsible mechanisms are, it is evident that the expression of c-*myc* is adversely regulated by PDGF and IFN-α in AIDS-KS spindle cells. It is important to know whether the regulation of c-*myc* expression may have an impact on the migration and proliferation of AIDS-KS spindle cells. The correlation between c-*myc* gene expression and proliferation is variable in different cell types.[18,24,35,38,39] In AIDS-KS spindle cells, c-*myc* expression was positively correlated with proliferation and migration, which suggested that the c-Myc protein may regulate proliferation and migration of these cells.

Specific inhibition of c-*myc* expression using antisense ODNs was carried out to investigate the role of the c-Myc protein in AIDS-KS spindle cells in more detail. PDGF-B-induced proliferation and migration were reduced by c-*myc*-specific asODN. Inhibition of migration was clearly stronger than inhibition of proliferation. These results are in agreement with the results observed by others[25] and may be explained by a better uptake of ODN into cells in suspension, as is the case in the Boyden chamber assay.

To exclude non-sequence-specific effects of ODN, which have been observed by others,[40,41] numerous controls were performed in this study. For the following reasons these experiments strongly suggest that in our experimental set-up asODN spe-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cifically inhibited c-*myc* expression. First, low concentrations of asODN (1 to 3 μmol/L) were effective in the inhibition of AIDS-KS cell migration and proliferation, whereas second, scrODN and sODN had no effect. Third, specific reduction of the c-Myc protein after incubation with asODN was shown. Fourth, synthesis of c-Fos, a protein unrelated to c-Myc, the expression of which is also induced by PDGF-B, was not affected by c-*myc*-specific asODN. Fifth, interaction of PDGF-B with its receptor has not been compromised by the ODN. This was demonstrated by 2-[^{3}H]deoxyglucose uptake, which was induced by PDGF-B, and was not affected by asODN, sODN, or scrODN. On the basis of these results, we conclude that down-regulation of c-*myc* expression is sufficient to inhibit proliferation and migration of AIDS-KS spindle cells. This indicates that c-Myc may be a major regulatory molecule of AIDS-KS spindle cell proliferation and migration *in vitro*.

In addition, high amounts of c-Myc protein were detected in the nuclei of KS spindle cells in all biopsy specimens from primary lesions of AIDS-KS patients (nine of nine), which provides evidence that c-*myc* may also regulate proliferation and migration in AIDS-KS spindle cells *in vivo*.

Adverse regulation of c-*myc* expression in AIDS-KS spindle cells by PDGF-B, a major KS pathogen, and by IFN-α, a major KS inhibitory factor, suggested that important molecular pathways of KS pathogenesis and therapy may be focused on the regulation of c-*myc* gene expression. Interestingly, bFGF, which has been heralded as a major regulator of KS angiogenesis recently,[6,34] induced c-*myc* expression in cultivated AIDS-KS spindle cells. In addition, we could show that inhibition of c-*myc* expression also inhibited bFGF-induced proliferation of AIDS-KS spindle cells (our unpublished data). It will be interesting to learn whether the activities of additional AIDS-KS pathogens, such as the HIV-Tat protein[6] and vascular endothelial growth factor,[42,43] also converge onto the regulation of c-*myc* gene expression in AIDS-KS spindle cells. This would further underline the importance of the proto-oncogene c-*myc* as a molecular target for medical treatment of AIDS-KS.

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References

1. Friedman-Kien A: Disseminated Kaposi's sarcoma syndrome in young homosexual men. J Am Acad Dermatol 1981, 5:468–471
2. Safai B, Johnson KG, Myskowski PL, Koziner B, Yang SY, Cunningham-Rundles S, Godbold JH, Dupont B: The natural history of Kaposi's sarcoma in the acquired immunodeficiency syndrome. Ann Intern Med 1985, 103:744–750
3. Stürzl M, Brandstetter H, Roth WK: Kaposi's sarcoma: a review of gene expression and ultrastructure of KS spindle cells *in vivo*. AIDS Res Hum Retroviruses 1992, 8:1753–1763
4. Roth WK, Brandstetter H, Stürzl M: Cellular and molecular features of HIV-associated Kaposi's sarcoma. AIDS 1992, 6:895–913
5. Ensoli B, Barillari G, Gallo RC: Pathogenesis of AIDS-associated Kaposi's sarcoma. Hematol Oncol Clin North Am 1991, 5:281–295
6. Ensoli B, Gendelman R, Markham P, Fiorelli V, Colombini S, Raffeld M, Cafaro A, Chang HK, Brady JN, Gallo RC: Synergy between basic fibroblast growth factor and human immunodeficiency virus type 1 Tat protein in induction of Kaposi's sarcoma. Nature 1994, 321: 674–680
7. Chang Y, Cesarman E, Pessin MS, Lee F, Culpepper J, Knowles DM, Moore PS: Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. Science 1994, 266:1685–1869
8. Stürzl M, Roth WK, Brockmeyer NH, Zietz C, Speiser B,

Figure 8. *Immunohistochemical staining for c-Myc protein on tissue sections of AIDS-KS biopsies. The staining was performed as described in Materials and Methods.* **A:** *Overview of a KS section stained for c-Myc protein. Aggregates of spindle cells are marked by an* arrow. **B:** *AIDS-KS of the angioma-like type with spindle cells positive for c-Myc protein* (arrow). **C:** *AIDS-KS of the fibrosarcomatous type with spindle cells positive for c-Myc protein* (arrow) *and negative inflammatory cells* (arrowhead). **D:** *Higher magnification demonstrated c-Myc positive* (arrow) *and negative* (arrowhead) *staining of nuclei of spindle cells.* **E:** *Reduced amounts of c-Myc protein were present in an early AIDS-KS lesion.* **G:** *Detection of c-Myc protein in proliferating basal cells of the epidermis overlaying KS.* **F** *and* **H:** *Negative controls, for which AIDS-KS sections were subjected to the immunohistochemical procedure omitting the primary antibody.*

Hofschneider PH: Expression of platelet-derived growth factor and its receptor in AIDS-related Kaposi's sarcoma *in vivo* suggests paracrine and autocrine mechanisms of tumor maintenance. Proc Natl Acad Sci USA 1992, 89:7046–7050

9. Stürzl M, Brandstetter H, Zietz C, Eisenburg B, Raivich G, Gearing DP, Brockmeyer NH, Hofschneider PH: Identification of interleukin-1 and platelet-derived growth factor-B as major mitogens for the spindle cells of Kaposi's sarcoma: a combined *in vitro* and *in vivo* analysis. Oncogene 1995, 19:2007–2016
10. Roth WK, Werner S, Schirren CG, Hofschneider PH: Depletion of PDGF from serum inhibits growth of AIDS-related and sporadic Kaposi's sarcoma cells in culture. Oncogene 1989, 4:483–487
11. Werner S, Hofschneider PH, Heldin CH, Östman A, Roth WK: Cultured Kaposi's sarcoma-derived cells express functional PDGF A-type and B-type receptors. Exp Cell Res 1990, 187:98–103
12. Lilenbaum RC, Ratner L: Systemic treatment of Kaposi's sarcoma: current status and future directions. AIDS 1994, 8:141–151
13. Stickler MC, Friedman-Kien AE: Kaposi's sarcoma. Clin Dermatol 1991, 9:39–47
14. Reiter Z, Ozes ON, Blatt LM, Stürzl M, Taylor MW: A possible role for interferon-a and activated natural killer cells in remission of AIDS-related Kaposi's sarcoma: *in vitro* studies. J Acquired Immune Defic Syndr 1992, 5:469–476
15. Singh RK, Gutman M, Bucana CD, Sanchez R, Liansa N, Fiedler IJ: Interferons α and β down-regulate the expression of basic fibroblast growth factor in human carcinomas. Proc Natl Acad Sci USA 1995, 92:4562–4566
16. Marcu KB, Bossone SA, Patel AJ: Myc function and regulation. Annu Rev Biochem 1992, 61:809–860
17. Kelly K, Cochran BH, Stiles CD, Leder P: Cell-specific regulation of the c-*myc* gene by lymphocyte mitogens and platelet-derived growth factor. Cell 1983, 35:603–610
18. Armelin HA, Armelin MCS, Kelly K, Stewart T, Leder P, Cochran BH, Stiles CD: Functional role for c-*myc* in mitogenic response to platelet-derived growth factor. Nature 1984, 310:655–660
19. Einat M, Resnitzky D, Kimchi A: Inhibitory effects of interferon on the expression of genes regulated by platelet-derived growth factor. Proc Natl Acad Sci USA 1985, 82:7608–7612
20. Einat M, Resnitzky D, Kimchi A: Close link between reduction of c-*myc* expression by interferon and G_0/G_1 arrest. Nature 1985, 313:597–600
21. Heikkila R, Schwab G, Wickstrom E, Loke SL, Pluznik DH, Watt R, Neckers LM: A c-*myc* antisense oligodeoxynucleotide inhibits entry into S phase but not progress from G_0 to G_1. Nature 1987, 328:445–449
22. Wickstrom EL, Bacon TA, Gonzalez A, Freeman DL, Lyman GH, Wickstrom E: Human promyelocytic leukemia HL-60 cell proliferation and c-*myc* protein expression are inhibited by an antisense pentadecadeoxynucleotide target against c-*myc* mRNA. Proc Natl Acad Sci USA 1988, 85:1028–1032
23. Bennett MR, Anglin S, McEwan JR, Jargoe R, Newby AC, Evan GI: Inhibition of vascular smooth muscle cell proliferation *in vitro* and *in vivo* by c-*myc* antisense oligodeoxynucleotides. J Clin Invest 1994, 93:820–828
24. Holt JT, Redner RL, Nienhuis AW: An oligomer complementary to c-*myc* mRNA inhibits proliferation of HL-60 promyelocytic cells and induces differentiation. Mol Cell Biol 1988, 8:963–973
25. Biro S, Fu YM, Yu ZX, Epstein SE: Inhibitory effects of antisense oligodeoxynucleotides targeting c-*myc* mRNA on smooth muscle cell proliferation and migration. Proc Natl Acad Sci USA 1993, 90:654–658
26. Roth WK, Werner S, Risau W, Remberger K, Hofschneider PH: Cultured, AIDS-related Kaposi's sarcoma cells express endothelial cell markers and are weakly malignant *in vitro*. Int J Cancer 1988, 42:767–773
27. Boyden S: The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leukocytes. J Exp Med 1962, 115:453–466
28. Sambrook J, Fritsch EF, Maniatis T: Molecular Cloning: A Laboratory Manual, ed 2. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press, 1989
29. Persson H, Henninghausen L, Taub R, DeGrado W, Leder P: Molecular genetic characterization of epitope-specific monoclonal antibodies against the Myc family. Science 1984, 225:687–693
30. Ikegaki N, Kennett R: Antibodies to human c-*myc* oncogene product: evidence of an evolutionary conserved protein induced during cell proliferation. Oncogene 1990, 5:397–403
31. Centers for Disease Control: 1993. Revised classification system for HIV infection and expanded surveilliance case definition for AIDS among adolescents and adults. MMWR Morb Mortal Wkly Rep 1992, 41:1–19
32. Klein ML, Bartley TD, Lai PH, Lu HS: Structural characterization of recombinant consensus interferon-α. J Chromatogr 1988, 454:205–215
33. Ozes ON, Reiter Z, Klein S, Blatt LM, Taylor M: A comparison of interferon-con1 with natural recombinant interferons-α: antiviral, antiproliferative, and natural killer-inducing activities. J Interferon Res 1992, 12:55–59
34. Albini A, Fontanini G, Masiello L, Tacchetti C, Bigini D, Luzzi P, Noonan DM, Stetler-Stevenson G: Angiogenic potential *in vivo* by Kaposi's sarcoma cell-free supernatants of HIV-1 *tat* product: inhibition of KS-like lesions by tissue inhibitor of metalloproteinase-2. AIDS 1994, 8:1237–1244
35. Chaouchi N, Wallon C, Taieb J, Auffredou MT, Tertian G, Lemoine FM, Delfraissy JF, Vazquez A: Interferon-α-mediated prevention of *in vitro* apoptosis of chronic lymphocytic leukemia B cells: role of *bcl-2* and c-*myc*. Clin Immunol Immunpathol 1994, 73:197–204
36. Hathorn RW, Tso CL, Kaboo R, Pang S, Figlin R, Sawyers C, deKernion JB, Belldegrun A: *In vitro* modulation of the invasive and metastatic potentials of human re-

nal cell carcinoma by interleukin-2 and/or interferon-α gene transfer. Cancer 1994, 74:1904–1911.
37. Dani C, Mechti N, Piechaczyk M, Lebleu B, Jeanteur P, Blanchard JM: Increased rate of degradation of c-*myc* mRNA in interferon-treated Daudi cells. Proc Natl Acad Sci USA 1985, 82:4896–4899
38. Womer R, Frick K, Mitchell CD, Ross AH, Bishayee S, Scher CD: PDGF induces c-*myc* mRNA expression in MG-63 human osteosarcoma cells but does not stimulate cell replication. J Cell Physiol 1987, 132:65–72
39. Ninomya-Tsuji J, Torti FM, Ringold GM: Tumor necrosis factor-induced c-*myc* expression in the absence of mitogenesis is associated with inhibition of adipocyte differentiation. Proc Natl Acad Sci USA 1993, 90:9611–9615
40. Wagner RW: Gene inhibition using antisense oligodeoxynucleotides. Nature 1994, 372:333–335
41. Burgess TL, Fisher EF, Ross SL, Bready JV, Qian Y-X, Bayewitch LA, Cohen AM, Herrera CJ, Hu SS-F, Kramer TB, Lott FD, Martin FH, Pierce GF, Simonet L, Farrel CL: The antiproliferative activity of c-*myb* and c-*myc* antisense oligonucleotides in smooth muscle cells is caused by a nonantisense mechanism. Proc Natl Acad Sci USA 1995, 92:4051–4055
42. Stürzl M, Cornali E, Blasig C, Bunk D, Schreier A: Growth factors, cytokines, and chemokines in Kaposi's sarcoma. Nouv Dermatol 1966, 15 (suppl 1): 8–14
43. Cornali E, Zietz C, Benelli R, Weninger W, Masiello L, Breier G, Tschachler E, Albini A, Stürzl M: Vascular endothelial growth factor regulates angiogenesis and vascular permeability in Kaposi's sarcoma. Am J Pathol 1996, 149:1851–1869