Journal of Cellular Biochemistry Supplement 36:162–169 (2001)

Zinc-α_2-Glycoprotein Hinders Cell Proliferation and Reduces *cdc2* Expression

Nonggao He,[1] Henry Brysk,[1] Stephen K. Tyring,[1,2] Iwao Ohkubo,[3] and Miriam M. Brysk[1,2,4]*

[1]Department of Dermatology, University of Texas Medical Branch, Galveston, Texas 77555
[2]Department of Microbiology & Immunology, University of Texas Medical Branch, Galveston, Texas 77555
[3]Department of Medical Biochemistry, Shiga University of Medical Science, Seta, Otsu 520-2192, Japan
[4]Department of Human Biological Chemistry & Genetics, University of Texas Medical Branch, Galveston, Texas 77555

Abstract Zinc-α_2-glycoprotein (Zn$\alpha_2$gp) is widely distributed in body fluids and epithelia. Its expression in stratified epithelia increases with differentiation. We previously showed that Zn $\alpha_2$gp has ribonuclease activity, and that squamous tumor cells grown on a matrix of Zn$\alpha_2$gp were growth-inhibited. Here we demonstrate, both by adding Zn$\alpha_2$gp to the culture medium and, more unequivocally, by stably transfecting SiHa cells with Zn$\alpha_2$gp cDNA, that the introduction of Zn$\alpha_2$gp into SiHa tumor cells reduces proliferation. In response to Zn$\alpha_2$gp, we find an accumulation of the cell population in G_2/M by flow cytometry, paralleling the reduction of proliferation. In order to distinguish growth inhibition by cell cycle arrest from that produced by apoptosis or differentiation, we examine by RT-PCR how Zn$\alpha_2$gp affects the expression of genes commonly used as markers of these properties. No changes are observed for PCNA, p53, c-*myc, or bcl-2.* Only *cdc2* expression responds to Zn$\alpha_2$gp, with a reduction of up to over a factor of two. *Cdc2* is the only cyclin-dependent kinase regulating the G_2/M transition without redundancy and is required as a rate-limiting step in the cell cycle. Its increased expression has been directly linked to increased proliferation and decreased differentiation of advanced tumors; conversely, its downregulation by Zn$\alpha_2$gp might hinder tumor progression. J. Cell. Biochem. Suppl. 36:162–169, 2001. © 2001 Wiley-Liss, Inc.

Key words: cell growth; growth arrest; cell cycle; SiHa cell line; transfection

Zinc-α_2-glycoprotein (Zn$\alpha_2$gp) was initially purified from plasma [Bürgi and Schmid, 1961]; it can be precipitated by adding zinc ions and it displays electrophoretic mobility in the α_2-region of the plasma globulins, hence its name. It has been detected in most body fluids [Poortmans and Schmid, 1968; Frenette et al., 1987; Ohkubo et al., 1990; Sánchez et al., 1992], in the secretory epithelia of various human glands [Tada et al., 1991], and in the epidermis [Lei et al., 1997]. The molecular weight is in the range 35–41 kDa, depending on variations in glycosylation among different tissues (mostly about 12–18% carbohydrate except that seminal plasma is unglycosylated) [Ohkubo et al., 1990]. The amino acid sequence consists of a single polypeptide chain of 278 amino acids [Araki et al., 1988]. The nucleotide sequence, ascribed to a single active gene and one or two pseudogenes, differs only in posttranslational modifications for prostate [Ueyama et al., 1993], breast [Freije et al., 1993], and epidermis [Lei et al., 1997]. The crystalline structure has been determined [Sánchez et al., 1997].

Notwithstanding the widespread occurrence of Zn$\alpha_2$gp in different body tissues and thorough knowledge of its structure, the clues to its function are fragmentary. Despite considerable homology with major histocompatibility complex chains, there is no evidence that it plays an immunological role. Zn$\alpha_2$gp has ribonuclease activity against single-stranded RNA and preferentially cleaves RNA at its pyrimidine residues [Lei et al., 1998]. A lipid-mobilizing factor associated with fat depletion and the wasting syndrome in cancer patients has been shown to have the same amino acid sequence as Zn$\alpha_2$gp [Todorov et al., 1998]. Different tumor cell lines attach and spread on a matrix of Zn$\alpha_2$gp to a varying extent [Takagaki et al., 1994; Lei et al., 1999].

Department of Dermatology, University of Texas Medical Branch, Galveston, Texas 77555-0783.Received 5 September 2000; Accepted 2 November 2000

© 2001 Wiley-Liss, Inc.
This article published online in Wiley InterScience, March 21, 2001.

Zn$\alpha_2$gp has received most attention (epidemiological, rather than mechanistic) as a putative clinical marker. Zn$\alpha_2$gp expression has been correlated with differentiation in various cell types. A significant association has been observed between Zn$\alpha_2$gp levels and the histological grade of breast cancer tumors, with higher levels found in well-differentiated tumors than in poorly differentiated ones [Díez-Itza et al., 1993]. Zn$\alpha_2$gp mRNA expression is lower in oral squamous cell carcinomas than in normal tissue; among the tumors, it is highest in the better differentiated ones [Brysk et al., 1999]; a similar trend pertains in comparing different kinds of epidermal tumors (with higher levels in normal epithelia than in squamous cell carcinomas or Merkel cell carcinomas, and higher in the latter than in basal cell carcinomas) [Lei et al., 1997]. In the normal epidermis, the Zn$\alpha_2$gp protein is expressed in the most differentiated cells (the granular and stratum corneum layers); it is not detected in the proliferating basal cells. Inhibition of proliferation is often associated with the induction of differentiation, particularly for epithelial cells (and vice versa). Psoriasis is a T cell-mediated inflammatory disease characterized by hyperproliferation and aberrant differentiation; Zn$\alpha_2$gp is not found in any epidermal cell layer of psoriatic epidermis [Chen et al., 2000].

MATERIALS AND METHODS

Cell Culture

The cervical SiHa squamous cell carcinoma cell line was derived from the American Type Culture Collection. The cells were maintained in Dulbecco's Minimal Essential Medium, supplemented with 10% fetal bovine serum, penicillin, and streptomycin, and grown in a 5% CO_2 incubator at 37°C. Tissue culture media and fetal calf serum were obtained from Gibco BRL Life Technologies (Grand Island, NY).

Cell Proliferation Assay

Cell proliferation was measured using a modified MTT [3-(4, 5 dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] (Sigma, St. Louis, MO) cytotoxicity assay [Mosmann, 1993]. Briefly, 3×10^3 cells in 100 µl aliquots of the growth medium were plated into 96-well flat-bottomed microtiter plates. The medium was replaced after 6 h of incubation at 37°C with 100 µl of fresh medium to which 20 µg/ml of recombinant Zn$\alpha_2$gp had been added. The control wells received the medium alone. After 24, 48, 72, and 96 h of incubation, 10 µl aliquots of MTT solution (2 mg/ml in PBS) were added to each well and the plates were incubated for a further 4 h. The medium was then removed and 10% DMSO in isopropanol was added at room temperature to solubilize the formazan crystals. The optical density (OD) of the wells was measured at a wavelength of 570 nm with, a Thermo Max microplate reader (Molecular Devices, Sunnyvale, CA). Five replicate wells were used for each assay point in three separate experiments.

Transfection of Cells With Zn$\alpha_2$gp cDNA

Full-length Zn$\alpha_2$gp cDNA for transfection was cloned in our laboratory from a human epidermal keratinocyte cDNA library [Lei et al., 1997]. The Zn$\alpha_2$gp cDNA was doubly digested with the restriction enzymes HindIII and XbaI, then cloned into the expression vector pcDNA 3.1(+) (Invitrogen, Carlsbad, CA) previously digested with the same enzymes. The empty vector pcDNA 3.1(+) and the vector containing Zn$\alpha_2$gp cDNA were used for transfection. Transfection was performed with the TransFast Transfection Reagent Kit (Promega, Madison, WI) according to the manufacturer's instructions. Stable transfectants were selected after culture of the cells for 2 weeks in the growth medium containing 400 µg/ml of G418. We have now grown the transfectants for some 6 months in the absence of G418 and the cells continue to express the Zn$\alpha_2$gp protein (on Western blots).

Western Blotting

Cells transfected with Zn$\alpha_2$gp or with the empty vector were homogenized in DPBS, and aliquots were analyzed by electrophoresis on 10% SDS-polyacrylamide gels. The gels were then transferred electrophoretically to a nitrocellulose membrane in a buffer of 20% methanol, 25 mM Tris–HCl, and 192 mM glycine. The blots were quenched in TBST (10 mM Tris–HCl, pH 8.8, 150 mM NaCl, 0.05% Tween 20) containing 3% non-fat dried milk, for 30 min, then washed twice for 10 min in the buffer alone. The blot was then incubated for 1 h with a 1:500 dilution of rabbit anti-prostate Zn$\alpha_2$gp IgG [Tada et al., 1991] in 0.5% dried milk in TBST, then washed twice for 10 min each with unsupplemented TBST. The blot was then incubated for 1 h in a 1:500 dilution

of peroxidase-conjugated sheep anti-rabbit IgG (Cappel, ICN Biomedicals, Costa Mesa, CA), and the color was developed by the ECL (Enhanced Chemiluminescence) kit (Amersham Life Science, Arlington Heights, IL).

Flow Cytometry

Cultured cells were detached with trypsin, harvested by centrifugation in culture medium, and washed twice in PBS. They were then fixed overnight in 70% ethanol at 4°C then suspended in fresh PBS. The fixed cell suspensions were incubated with RNase I (1 mg/ml) at 37°C for 30 min; then propidium iodide was added to a concentration of 50 μg/ml. The cells were analyzed using a Becton Dickinson FACS SCAN.

Quantitative Polymerase Chain Reaction Analysis

Total RNA was isolated from the cells using ultra-pure Trizol Reagent (Gibco BRL). Quantitative RT-PCR was performed using a Clontech Advantage RT-for-PCR Kit and the Advantage 2 PCR Enzyme System method (Clontech, Palo Alto, CA) according to the manufacturer's instructions. The cDNA for the entire population of mRNA molecules was first synthesized in a 20 ml reaction volume by reverse transcriptase using an olig(dT) primer, at 42°C for 1 h. The cDNA product was then diluted to 100 μl, and 5 ml aliquots of the diluent were used for cDNA amplification. The cDNA amplification was carried out in a 50 μl reaction mixture for 30 cycles with the specific sense and anti-sense primers of cell-cycle-related genes, including the standard G3PDH as internal control. Each cycle comprised 30 s at 95°C for denaturation, 3 min at 68°C for annealing, and 3 min at 68°C for extension. The PCR products were resolved on 1.5% agarose gels and visualized under ultraviolet light. The bands were photographed using an Alpha Imager 2000 (Alpha Innotech, San Leandro, CA) and band intensities were estimated using the Alpha Ease 3.3 software.

RESULTS

Zn$\alpha_2$gp is Expressed in Transfected Cells

SiHa cells do not normally express the Zn$\alpha_2$gp protein. We transfected SiHa cells with Zn$\alpha_2$gp cDNA using the pcDNA 3.1(+) vector, and have now established a stable continuous cell line that synthesizes the Zn$\alpha_2$gp protein; it continues to express Zn$\alpha_2$gp some 6 months after the transfection, producing 2–4 μg of Zn$\alpha_2$gp per mg of cell protein. To confirm the presence of endogenous Zn$\alpha_2$gp, we homogenized the transfected cells and demonstrated Zn$\alpha_2$gp expression by Western blotting. In contrast, homogenates of cells transfected with the empty vector did not express Zn$\alpha_2$gp. Zn$\alpha_2$gp produced by the transfected cell line has a slightly lower molecular weight than Zn$\alpha_2$gp isolated from body fluids, probably reflecting posttranslational modifications (Fig. 1).

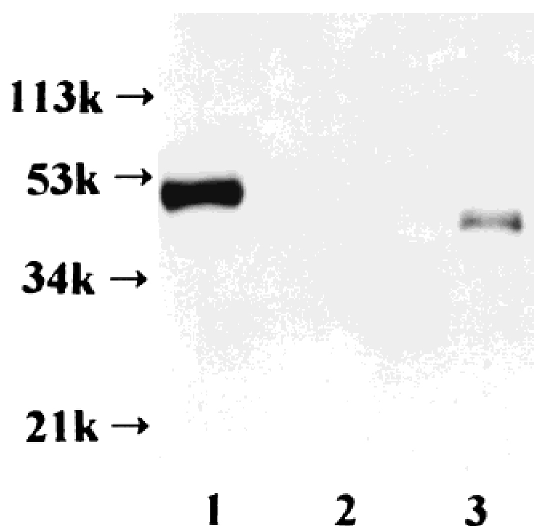

Fig. 1. Western blots for Zn$\alpha_2$gp expression. **Lane 1:** Zn$\alpha_2$gp isolated from body fluids. **Lane 2:** homogenates of cells transfected with the empty vector. **Lane 3:** homogenates of cells transfected with Zn$\alpha_2$gp.

Zn$\alpha_2$gp Inhibits Cell Proliferation

We investigated the effect of Zn$\alpha_2$gp on cell proliferation by an MTT assay (Fig. 2), comparing the proliferation of untreated SiHa cells (as controls) with the proliferation of cells transfected with Zn$\alpha_2$gp cDNA, or incubated with recombinant Zn$\alpha_2$gp (20 μg/ml) or both. Results at 10 μg/ml were marginal (not shown). Both these concentrations are within physiological limits. The assay results are displayed in Figure 2A; for convenience, they are also replotted as ratios of numbers of treated-to-untreated cells (Fig. 2B). Transfection of SiHa cells with Zn$\alpha_2$gp reduced the proliferation to about 65% of the control cells after 4 days. For untransfected SiHa cells incubated with exogenous Zn$\alpha_2$gp, the corresponding reduced proliferation was 45%. There was further only a small increase in

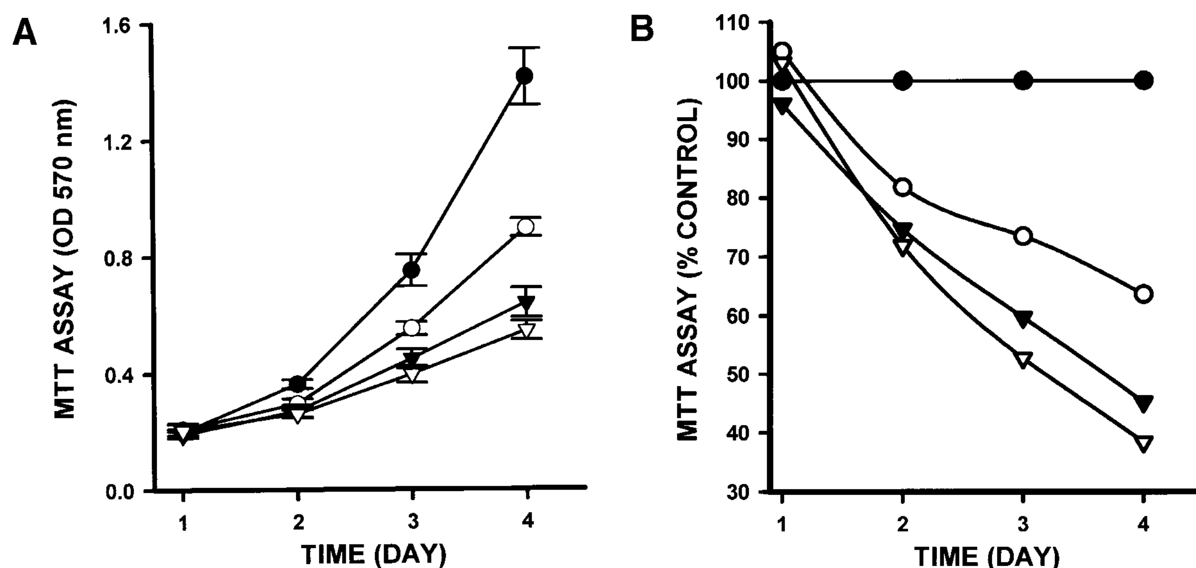

Fig. 2. Effect of Zn$\alpha_2$gp on cell proliferation, by MTT assay. **A:** Growth curves for: (filled circles) untreated SiHa cells (controls); (open circles) cells transfected with Zn$\alpha_2$gp cDNA; (filled triangles) untransfected cells incubated with recombinant Zn$\alpha_2$gp (20 µg/ml); (open triangles) transfected cells incubated with Zn$\alpha_2$gp. **B:** Same data replotted as ratio to controls. Note that time scale does not start at 0.

growth inhibition for transfectants as against untransfected cells both treated with exogenous Zn$\alpha_2$gp. The cells were viable in all cases, as verified by trypan-blue exclusion.

Zn$\alpha_2$gp Arrests the Cell Cycle at the G2/M Phase

We examined cell populations by flow cytometry to ascertain at what stage of the cell cycle Zn$\alpha_2$gp impacts proliferation (Fig. 3). The exposure to Zn$\alpha_2$gp resulted in an accumulation of cells at the G_2/M interface. After 2 days of culture, roughly 14% of the control cells were in the G_2/M phase, as against 20% of the Zn$\alpha_2$gp transfectants and 24% of the exogenously Zn$\alpha_2$gp-treated cells.

Expression of Markers Associated With Proliferation

We examined, by RT-PCR, the expression of genes associated with proliferation and apoptosis (Fig. 4). Zn$\alpha_2$gp had no effect on the mRNA levels of PCNA, p53,c-myc, or bcl-2. Only cdc2 expression was affected; it diminished. For greater clarity, the cdc2 band intensities are displayed in Figure 5 relative to the expression of the controls. In the cells either transfected or treated with Zn$\alpha_2$gp, cdc2 expression is reduced by about 25–30%; in the cells both transfected and treated, it is diminished by 55%.

DISCUSSION

Zn$\alpha_2$gp is a positive marker for differentiation of breast [Díez-Itza et al., 1993], epidermal [Lei et al., 1997], and oral [Brysk et al., 1999] tumors. Differentiation is often (though not inevitably) associated with growth arrest, particularly for epithelial cells. This led us to suspect that Zn$\alpha_2$gp might affect cell proliferation. Consistently, although the Zn$\alpha_2$gp protein is found in the outermost (most differentiated) layers of normal human epidermal cells, it is not seen at all in psoriatic cells (which evidence hyperproliferation and aberrant differentiation) [Chen et al., 2000]. Consequently, while characterizing the attachment of a squamous carcinoma cell line to Zn$\alpha_2$gp acting as a matrix protein, we measured cell proliferation. There was a total inhibition of growth for the first 2 days; thereafter, proliferation resumed but at a lower rate [Lei et al., 1999]. These were the first published observations of a direct impact of Zn$\alpha_2$gp on cell proliferation. They left undetermined where and how Zn$\alpha_2$gp acted, whether through a cell-surface binding effect or after penetration inside the cell.

The first purpose of the present study was to demonstrate that the introduction of Zn$\alpha_2$gp into the cells results in reduced proliferation, by incubation of the cells with Zn$\alpha_2$gp and, more

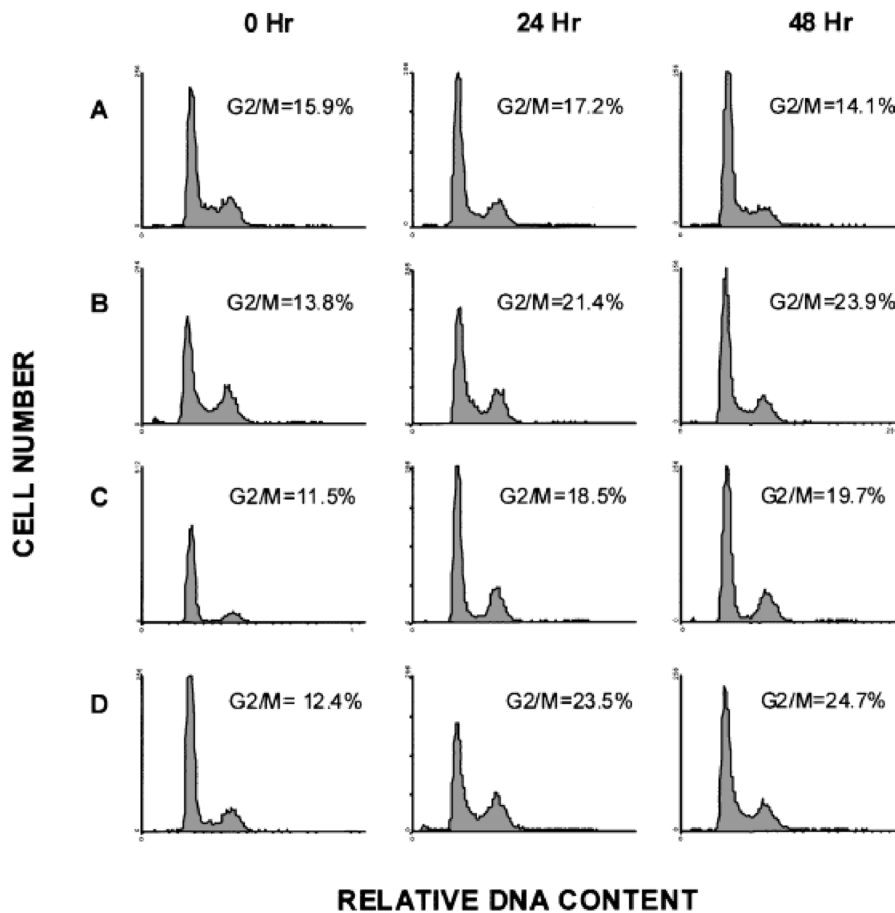

Fig. 3. Cell Cycle phase distributions by flow cytometry. **A:** untreated SiHa cells (controls); **B:** untransfected cells incubated with recombinant Zn$\alpha_2$gp (20 µg/ml); **C:** cells transfected with Zn$\alpha_2$gp cDNA; **D:** transfected cells incubated with Zn$\alpha_2$gp.

unequivocally, by transfection of the cells with Zn$\alpha_2$gp cDNA. The SiHa human cervical epithelial squamous cell carcinoma cell line does not normally express the Zn$\alpha_2$gp protein. We transfected SiHa cells with Zn$\alpha_2$gp cDNA and established a cell line which continues to express the Zn$\alpha_2$gp protein some 6 months after its transfection; it produces 2–4 µg of Zn$\alpha_2$gp/mg of cell protein.

We have shown here that Zn$\alpha_2$gp slows the proliferation of the SiHa cell line (Table I). It does so whether the cells are transfected with Zn$\alpha_2$gp cDNA or Zn$\alpha_2$gp is added exogenously to the culture medium. At 20 µg/ml of culture medium, exogenous Zn$\alpha_2$gp induced greater reduction of proliferation than did transfection with Zn$\alpha_2$gp cDNA, presumably indicating that more Zn$\alpha_2$gp entered the cells during incubation than was endogenously produced by transfection. Compounding transfection with incubation with Zn$\alpha_2$gp resulted in little additional reduction of proliferation, suggesting that Zn$\alpha_2$gp impacted proliferation by the same pathway in either mode of introduction of Zn$\alpha_2$gp and that we approached saturation of this effect.

What stage of the cell cycle is affected? We found by flow cytometry an increase of the SiHa cell population in the G2/M phase upon Zn$\alpha_2$gp enrichment (whether by transfection or by exogenous treatment). The accumulation of cells in G2/M paralleled the reduction of proliferation: it was highest for transfected cells treated with Zn$\alpha_2$gp, a bit less for control cells treated with Zn$\alpha_2$gp, still less for transfected cells not treated with Zn$\alpha_2$gp, and least for the untreated controls (Table I).

In order to distinguish inhibition of proliferation by cell-cycle arrest from that produced by apoptosis or differentiation, we examined by RT-PCR the expression of genes in common use as markers of proliferation, differentiation, and apoptosis. Gene expression of PCNA, a nuclear protein whose expression is correlated with the

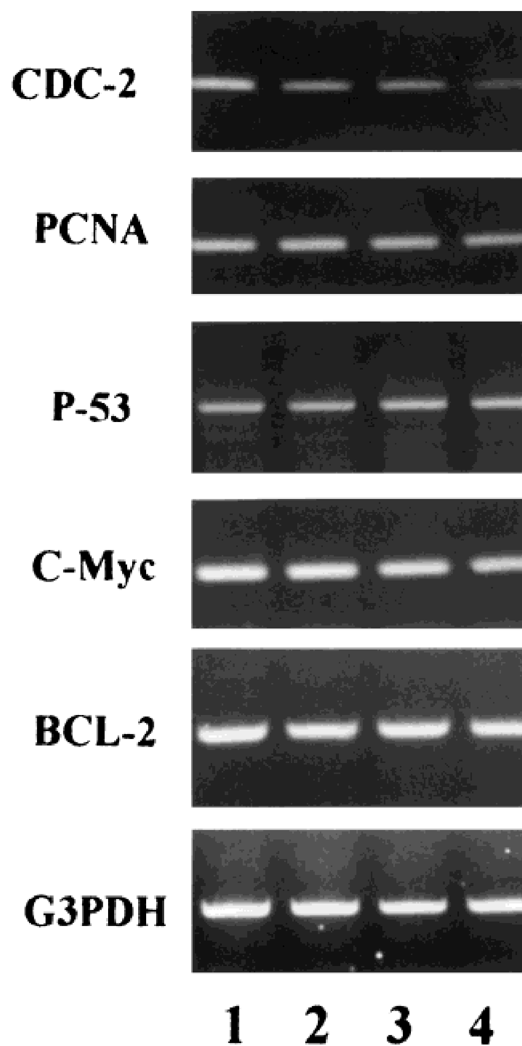

Fig. 4. Gene expression of markers of proliferation and apoptosis, by RT-PCR, as indicated (including G3PDH as control). **Lane 1:** untreated SiHa cells (controls). **Lane 2:** untransfected cells incubated with recombinant Zn$\alpha_2$gp (20 μg/ml). **Lane 3:** cells transfected with Zn$\alpha_2$gp cDNA. **Lane 4:** transfected cells incubated with Zn$\alpha_2$gp.

S-phase of the cell cycle [Celis and Celis, 1985], is not affected by Zn$\alpha_2$gp. We do not observe any alteration of p53 expression when the cells are treated with Zn$\alpha_2$gp. The tumor suppressor p53 and its mutants are oppositely associated with apoptosis (and concomitant changes in proliferation) in the late G1 stage [Donehower and Bradley, 1993]. The overexpression of wild type p53 in tumor cells can result in suppression of proliferation, induction of apoptosis or induction of differentiation. In vitro, levels of p53 appear to be downregulated in cells induced to differentiate. As with p53, we see no alteration of c-*myc* expression in cells treated with Zn$\alpha_2$gp.

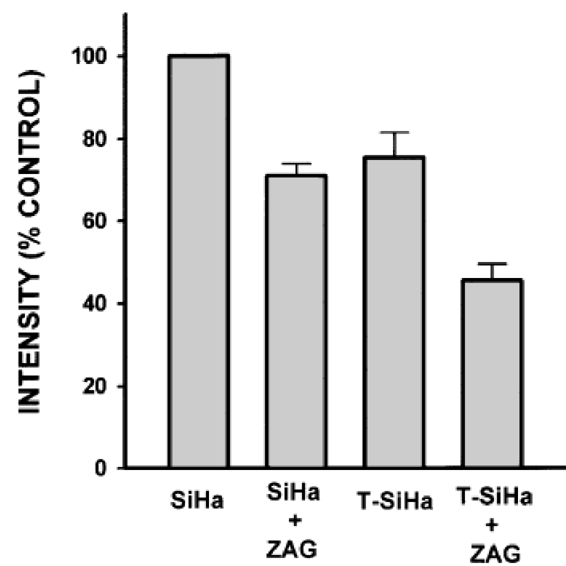

Fig. 5. Gene expression of *cdc2* in SiHa cells transfected and/or treated with Zn$\alpha_2$gp, relative to the expression of the untreated controls (from densitometer scans of data shown in Fig. 4).

The c-*myc* oncogene is a regulator of cell growth and differentiation which is activated in human cancer. Its enforced expression drives quiescent cells into the S-phase of the cell cycle when they are cultured in the absence of growth factors. Induction of inappropriate cell cycle progression by c-*myc* is accompanied by induction of apoptosis [Evan et al., 1994]. These opposite roles of c-myc in cell growth and cell death may reflect its ability to modify the expression of *bcl-2*; apoptotic cell death induced by c-myc is inhibited by *bcl-2* [Bissonnette et al., 1992]. The effects of *bcl-2* on cell proliferation and apoptosis are separable [Huang et al., 1997]. The *bcl-2* protein phosphorylates cdc2 during the G_2/M phase transition, leading to growth arrest without apoptosis [Furukawa et al., 2000]. The expression of *bcl-2* is unaffected by Zn$\alpha_2$gp.

TABLE I. Impact Upon SiHa Cells of Incubation With Recombinant Zn$\alpha_2$gp and of Transfection With Zn$\alpha_2$gp cDNA

	% Inhibition of cell growth	% Increase in G_2/M cells	% Increase of cdc2 mRNA
No Zn$\alpha_2$gp (control)	0	0	0
Incubation	55	69	38.5
Transfection	36	62	34.5
Transfection+Incubation	62	108	60.7

Only *cdc2* showed a response to Zn$\alpha_2$gp, a reduction of up to over a factor of two (Table I), again with the greatest effect for the cells both transfected and exogeneously treated. *cdc-2* kinase is a key regulator of the cell cycle. It is the only cyclin-dependent kinase specifically regulating only the G2/M transition [Ito et al., 2000] and is required as a rate-limiting step in the cell cycle [Draetta, 1990]. The *cdc2* gene encodes a 34 kDa protein kinase catalytic subunit ($p34^{cdc2}$) that is required for the transition from DNA replication in S phase to entry into mitosis (M phase). Levels of both *cdc2* and $p34^{cdc2}$ decline during differentiation [Norbury and Nurse, 1989]. Although transcriptional control is important in determining the level of *cdc2*, other forms of control such as regulation of mRNA stability may also be involved [Dalton, 1992]. Endogenous ribonucleases impact the quantity and stability of RNA. We have reported that Zn$\alpha_2$gp has ribonuclease activity which preferentially cleaves ssRNA substrates and is pyrimidine-specific [Lei et al., 1998].

In normal epidermal keratinocytes, Zn$\alpha_2$gp gene expression has been correlated with markers of cellular differentiation [Brysk et al., 1997]. The Zn$\alpha_2$gp protein (referred to as desquamin until its recent identification with Zn$\alpha_2$gp) was extracted from the stratum corneum of normal human epidermis [Brysk and Rajaraman, 1992] but could not be detected in cultured cells, whose less advanced differentiation does not extend to completion of the stratum corneum. The incubation of such cells with Zn$\alpha_2$gp in an optimal medium extended their maturation to the disappearance of nuclei and the formation of large squames [Selvanayagam et al., 1998]. The proliferation effects reported in the present study occur much earlier in the cell cycle than the stage at which the Zn$\alpha_2$gp protein is detected in normal epidermis, let alone a tumor cell line. There is no indication that adding Zn$\alpha_2$gp impacts the early stage of differentiation at that point in the cell cycle (*p53* and c-*myc* are both unaffected). This study does not model the role of endogenous Zn$\alpha_2$gp in cell development. It was motivated by interest in the potential of Zn$\alpha_2$gp (at a dosage within physiological limits in normal tissue) as an antiproliferative tumor agent.

Our results show that Zn$\alpha_2$gp slows cell proliferation, apparently without involving apoptosis or differentiation. There is no evidence that Zn$\alpha_2$gp affects proliferation before the G2 phase; its impact seems to be associated with G_2/M transition. Relatively high levels of Zn$\alpha_2$gp are found in body fluids (1–100 μg/ml) and in normal epithelia (1–75 μg/g). The expression of Zn$\alpha_2$gp is much lower in tumors than in their normal tissue counterparts. Perhaps, Zn$\alpha_2$gp functions as an antitumor surveillance agent that slows tumor cell growth. Arresting damaged cells at the G2/M transition may diminish cell division in those cells that contain mutations or DNA damage. Interestingly, *cdc2* is strongly upregulated with increased malignancy [Yamamoto et al., 1998; Ito et al., 2000]; conversely, its downregulation by Zn$\alpha_2$gp might hinder tumor progression.

REFERENCES

Araki T, Gejyo F, Takagaki K, Haupt H, Schwick HG, Bürgi W, Marti T, Schaller J, Rickli E, Brossmer R, Atkinson PH, Putnam FW, Schmid K. 1988. Complete amino acid sequence of human plasma Zn-α_2-glycoprotein and its homology to histocompatibility antigens. Proc Natl Acad Sci USA 85:679–683.

Bissonnette RP, Echeverri F, Mahboubi A, Green DR. 1992. Apoptotic cell death induced by c-*myc* is inhibited by *bcl-2*. Nature 359:552–556.

Brysk MM, Lei G, Adler-Storthz K, Chen Z, Brysk H, Tyring SK, Arany I. 1999. Zn-α_2-glycoprotein expression as a marker of differentiation in human oral tumors. Cancer Lett 137:117–120.

Brysk MM, Lei G, Rajaraman S, Selvanayagam P, Rassekh CH, Brysk H, Tyring SK, Arany I. 1997. Gene expression of zinc-α_2-glycoprotein in normal human epidermal and buccal epithelia. In Vivo 11:271–274.

Brysk MM, Rajaraman S. 1992. Cohesion and desquamation of epidermal stratum corneum. Prog Histochem Cytochem 25:1–55.

Bürgi W, Schmid K. 1961. Preparation and properties of Zn-α_2-glycoprotein of normal human plasma. J Biol Chem 236:1066–1074.

Celis JE, Celis A. 1985. Cell cycle dependent variations in the distribution of the nuclear protein cyclin proliferating cell nuclear antigen in cultured cells: subdivision of S phase. Proc Natl Acad Sci USA 82:3262–3266.

Chen SH, Arany I, Apisarnthanarax N, Rajaraman S, Tyring SK, Horikoshi T, Brysk H, Brysk MM. 2000. Response of keratinocytes from normal and psoriatic epidermis to interferon-γ differs in the expression of zinc-α_2-glycoprotein and cathepsin D. FASEB J 14:565–571.

Dalton S. 1992. Cell cycle regulation of the human *cdc2* gene. EMBO J 11:1797–1804.

Díez-Itza I, Sánchez LM, Allende MT, Vizoso F, Ruibal A, López-Otín C. 1993. Zn-α_2-glycoprotein levels in breast cancer cytosols and correlation with clinical, histological and biochemical parameters. Eur J Cancer 29A:1256–1260.

Donehower LA, Bradley A. 1993. The tumor suppressor p53. Biochim Biophys Acta 1155:181–205.

Draetta G. 1990. Cell cycle control in eukaryotes: molecular mechanisms of cdc2 activation. Trends Biochem Sci 15:378–383.

Evan G, Harrington E, Fanidi A, Land H, Amati B, Bennett M. 1994. Integrated control of cell proliferation and cell death by the c-*myc* oncogene. Philos Trans R Soc Lond B Biol Sci 345:269–275.

Freije JP, Fueyo A, Uría JA, Velasco G, Sánchez LM, López-Boado YS C, López-Otín C. 1993. Human Zn-α_2-glycoprotein: complete genomic sequence, identification of a related pseudogene and relationship to class I major histocompatibility complex genes. Genomics 18:575–587.

Frenette G, Dubétien M, Tremblay RR. 1987. The major 40-kDa glycoprotein in human prostatic fluid is identical to Zn-α_2-glycoprotein. Prostate 11:257–270.

Furukawa Y, Iwase S, Kikuchi J, Terui Y, Nakamura M, Yamada H, Kano Y, Matsuda M. 2000. Phosphorylation of Bcl-2 protein by CDC2 kinase during G2/M phases and its role in cell cycle regulation. J Biol Chem 275:21661–21667.

Huang DC, O'Reilly LA, Strasser A, Cory S. 1997. The anti-apoptosis function of Bcl-2 can be genetically separated from its inhibitory effect on cell cycle entry. EMBO J 6(15):4628–4638.

Ito Y, Takeda T, Sakon M, Monden M, Tsujimoto M, Matsuura N. 2000. Expression and prognostic role of cyclin-dependent kinase 1 (cdc2) in hepatocellular carcinoma. Oncology 59:68–74.

Lei G, Arany I, Selvanayagam P, Rajaraman S, Ram S, Brysk H, Tyring SK, Brysk MM. 1997. Detection and cloning of epidermal zinc-α_2-glycoprotein cDNA and expression in normal human skin and in tumors. J Cell Biochem 67:216–222.

Lei G, Arany I, Tyring SK, Brysk H, Brysk MM. 1998. Zinc-α_2-glycoprotein has ribonuclease activity. Arch Biochem Biophys 355:160–164.

Lei G, Brysk H, Arany I, Tyring SK, Srinivasan G, Brysk MM. 1999. Characterization of zinc-α_2-glycoprotein as a cell adhesion molecule that inhibits the proliferation of an oral tumor cell line. J Cell Biochem 75:160–169.

Mosmann T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65:55–63.

Norbury CJ, Nurse P. 1989. Control of the higher eukaryote cell cycle by p34^{cdc2} homologues. Biochim Biophys Acta 989:85–95.

Ohkubo I, Niwa M, Takashima A, Nishikimi N, Gasa S, Sasaki M. 1990. Human seminal plasma Zn-α_2-glycoprotein: its purification and properties as compared with human plasma Zn-α_2-glycoprotein. Biochim Biophys Acta 1034:152–156.

Poortmans JR, Schmid K. 1968. The level of Zn-α_2-glycoprotein in normal body fluids and kidney extract. J Lab Clin Med 71:807–811.

Sánchez LM, López-Otín C, Bjorkman PJ. 1997. Biochemical characterization and crystalization of human Zn-α_2-glycoprotein, a soluble class I major histocompatibility complex homolog. Proc Natl Acad Sci USA 94:4626–4630.

Sánchez LM, Vizoso F, Díez-Itza I, López-Otín C. 1992. Identification of the major protein 1997 components in breast secretions from women with benign and malignant breast diseases. Cancer Res 52:95–100.

Selvanayagam P, Lei G, Bell T, Ram S, Brysk H, Rajaraman S, Brysk MM. 1998. Desquamin is an epidermal ribonuclease. J Cell Biochem 68:74–82.

Tada T, Ohkubo I, Niwa M, Sasaki M, Tateyama H, Eimoto T. 1991. Immunohistochemical localization of Zn-alpha2-glycoprotein in normal human tissues. J Histochem Cytochem 39:1221–1226.

Takagaki M, Honke K, Tsukamoto T, Higashiyama S, Taniguchi N, Makita A, Ohkubo I. 1994. Zn-α_2-glycoprotein is a novel adhesive protein. Biochem Biophys Res Comm 201:1339–1347.

Todorov PT, McDevitt TM, Meyer DJ, Ueyama H, Ohkubo I, Tisdale MJ. 1998. Purification and characterization of a tumor lipid-mobilizing factor. Cancer Res 58:2353–2358.

Ueyama H, Deng H-X, Ohkubo I. 1993. Molecular cloning and chromosomal assignment of the gene for human Zn-α_2-glycoprotein. Biochemistry 32:12968–12976.

Yamamoto H, Monden T, Miyoshi H, Izawa H, , Ikeda K, , Tsujie M, , Ohnishi T, , Sekimoto M, Tomita N, Monden M. 1998. Cdk2/cdc2 expression in colon carcinogenesis and effects of cdk2/cdc2 inhibitor in colon cancer cells. Int J Oncol 13:233–239.