Human Osteosarcoma Cells Resistant to Detachment by an Arg-Gly-Asp–containing Peptide Overproduce the Fibronectin Receptor

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Abstract. MG-63 human osteosarcoma cells were selected for attachment and growth in the presence of increasing concentrations of a synthetic peptide containing the cell attachment-promoting Arg-Gly-Asp sequence derived from the cell-binding region of fibronectin. Cells capable of attachment and growth in 5-mM concentrations of a peptide having the sequence Gly-Arg-Gly-Asp-Ser-Pro overproduce the cell surface receptor for fibronectin. In contrast, these cells show no differences in the numbers of vitronectin receptor they express as compared with the parental MG-63 cells. In agreement with the resistance of the selected cells to detachment by the peptide, 25-fold more Arg-Gly-Asp–containing peptide is required to prevent the attachment of these cells to fibronectin-coated surfaces than is needed to inhibit the attachment of MG-63 cells to the same substrate. However, similar concentrations of this peptide inhibit attachment of both cell lines to vitronectin-coated surfaces. The increase in fibronectin receptor is due to an increase in the levels of mRNA encoding the fibronectin receptor. Because of the nature of the selection process, we reasoned that this increase might be due to amplification of the fibronectin receptor gene, but no increase in gene copy number was detected by Southern blot analysis. The peptide-resistant cells display a very different morphology from that of the MG-63 cells, one that has a greater resemblance to that of osteocytes. The resistant cells also grow much more slowly than the MG-63 cells. The increased fibronectin receptor and altered morphology and growth properties were stable for at least 3 mo in the absence of peptide. The enhanced expression of the fibronectin receptor on the resistant cells indicates that cells are capable of altering the amount of fibronectin receptor on their surface in response to environmental factors and that this may in turn affect the phenotypic properties of the cell.

ADHESION of cells to extracellular matrix components appears to be fundamental in cell behavior patterns such as cell division, cell differentiation, and embryonic cell migration and sorting. Tumor invasion and metastasis may also occur due to altered mechanisms of adhesion of cells to the extracellular matrix (Ruoslahti, 1984; Roos, 1984; Liotta, 1986). Fibronectin is a well characterized component of the extracellular matrix and promotes cell attachment and spreading (Klebe, 1974; Pearlstein et al., 1976; Rubin et al., 1979; Ruoslahti and Hayman, 1979; Grinnell, 1980; Hynes and Yamada, 1982) as well as a variety of changes in cellular behavior (Ali and Hynes, 1978; Pennypacker et al., 1979; Loring et al., 1982; Yamada, 1983; Ruoslahti et al., 1985). The cell-binding domain of fibronectin contains the sequence L-arginyl-glycyl-L-aspartic acid (RGD) which can account for the ability of cells to specifically recognize and bind to fibronectin (Pierschbacher et al., 1983; Pierschbacher and Ruoslahti, 1984*a*, *b*). Synthetic peptides containing this sequence promote cell attachment when used to coat plastic surfaces and inhibit the attachment of cells to fibronectin-coated surfaces when presented in a soluble form (Pierschbacher and Ruoslahti, 1984*a*; Yamada and Kennedy, 1984). Thus, various cultured cell lines become detached from the substratum and their growth is arrested in the presence of such active peptides (Hayman et al., 1985). In vivo such peptides have also been found to inhibit gastrulation in amphibian embryos and neural crest cell migration in avian embryos (Boucaut et al., 1984) and to reduce dissemination of malignant cells to lungs in experimental metastasis (Humphries et al., 1986).

A cell surface receptor interacting with fibronectin in an RGD-dependent manner has recently been isolated from human osteosarcoma cells (Pytela et al., 1985*a*). Other adhesion-promoting molecules such as the collagens, laminin, and vitronectin are also present in the extracellular matrices of cells and contribute significantly to cell adhesion, some apparently via similar mechanisms, since some collagens (Bernard et al., 1983) and vitronectin (Suzuki et al., 1985) also contain the RGD sequence, and interact with their own specific RGD-directed receptors (Pytela et al., 1985*b*; Dedhar et al., 1987). Moreover, a chicken adhesion receptor interacts with fibronectin and laminin via an RGD-dependent mechanism (Hasegawa et al., 1985; Horwitz et al., 1985). One could predict, therefore, that selection of cells with the ability to attach and grow in the presence of synthetic peptides containing the RGD sequence should result in cell lines

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with either a quantitative or qualitative alteration in one or more of these receptors. Cell lines with such an altered phenotype would be useful in the further analysis of receptor–ligand interactions and also in increasing our understanding of the physiological role(s) of these receptors.

We report here that culturing MG-63 human osteosarcoma cells in increasingly high concentrations of a synthetic peptide, Gly-Arg-Gly-Asp-Ser-Pro (GRGDSP), results in cells capable of attaching and spreading in culture in the presence of high concentrations of this peptide. These cells overproduce fibronectin receptors but not vitronectin receptors and are morphologically different from the parental MG-63 cells.

Materials and Methods

Materials

Protein A Sepharose and FITC-conjugated goat anti-rabbit IgG were purchased from Sigma Chemical Co. (St. Louis, MO). ^{125}I-sodium iodide, ^{14}C-methylated molecular weight standards, and [^{32}P]dCTP were purchased from New England Nuclear (Boston, MA). Chemicals used for SDS-PAGE were from Bio-Rad Laboratories (Richmond, CA). Anti-HLA antiserum was the kind gift of Dr. David Cheresh, Scripps Research Institute, La Jolla, CA. Anti-epidermal growth factor receptor antibody was a kind gift from Dr. E. Adamson of this institution. Peptides were synthesized using a peptide synthesizer with the chemistry provided by the manufacturer (model 430A; Applied Biosystems, Inc., Foster City, CA).

Cells and Growth Conditions

Human osteosarcoma (MG-63) cells (Billiau et al., 1977) were obtained from American Type Culture Collection (Rockville, MD). The cells were cultured in DME, supplemented with 10% heat-inactivated FBS (Tissue Culture Biologicals, Tulare, CA), glutamine (2 mM), penicillin (100 U/ml), and streptomycin (100 μg/ml) (Irvine Scientific, Santa Ana, CA). For routine subculturing cell monolayers were washed with PBS (150 mM NaCl, 10 mM sodium phosphate, pH 7.3), and detached with EDTA (1 mM).

MG-63 cells were cultured in the presence of GRGDSP and GRGESP peptides as discussed in Results. The peptides were dissolved in DME and the pH was adjusted to 7.0 with sodium bicarbonate (7.5%). The solutions were then filter-sterilized before use.

Cell Staining and Flow Cytometric Analysis

Cell preparations were stained by indirect immunofluorescence and analyzed using a fluorescence-activated cell sorter (Cytofluorograf 50H with 2150 computer system, Ortho Diagnostic Systems Inc., Westwood, MA). The primary antibodies used were affinity-purified rabbit polyclonal anti-MG-63 vitronectin receptor and anti-MG-63 fibronectin receptor antibodies, rabbit anti-mouse epidermal growth factor receptor antiserum, and anti-HLA antibody. The second antibody used was FITC-conjugated goat anti-rabbit IgG. FITC fluorescence was detected as green fluorescence. Propidium iodide was used to label nonviable cells and detected as red fluorescence. Cell size was estimated by forward light scatter.

Immunoprecipitation

MG-63 and peptide-resistant variant cells were detached from culture with EDTA (1 mM) and resuspended with PBS containing $CaCl_2$ (1 mM) and $MgCl_2$ (1 mM). The cells (~2 × 10^6) were surface-labeled with ^{125}I as described previously (Pytela et al., 1985a; Lebien et al., 1982) and lysed in PBS containing SDS (0.1%), Triton X-100 (0.5%), sodium deoxycholate (0.5%), and PMSF (1 mM) for 15 min at 4°C. The solubilized cells were cleared of debris by centrifugation and cell extracts containing equivalent amounts of ^{125}I radioactivity were immunoprecipitated with the appropriate antibodies by coprecipitation with protein A Sepharose. The antigen–antibody complex was dissociated by boiling in sample buffer (200 mM Tris-HCl, pH 6.8 containing 3% SDS, 10% glycerol, and 0.001% bromphenol blue). Samples were analyzed by electrophoresis under reducing (5% 2-mercaptoethanol) or nonreducing conditions in 7.5% SDS-polyacrylamide gels (Laemmli, 1970) followed by autoradiography.

RNA Dot Blot and Southern Blot Analysis

Total cellular RNA was prepared by the guanidinium/cesium chloride method of Ullrich et al. (1977) as described by Maniatis et al. (1982). The RNA solution was adjusted to 20 mM sodium phosphate, pH 6.8, in 50% formamide (7% CV/V) formaldehyde and incubated at 65°C for 15 min to denature the RNA. Dilutions were then made in 11× SSC (1× SSC is 0.15 M NaCl, 1.5 mM sodium citrate) containing 37% formaldehyde and the RNA was applied to nitrocellulose filters that had been rinsed in 20× SSC. After baking at 80°C for 2 h in a vacuum oven, the filters were probed with [^{32}P]cDNAs as described below for the Southern blot analysis.

For restriction enyzme analysis, the high molecular weight DNAs were isolated from cells by the procedures of Blin and Stafford (1976). The restriction endonucleases were purchased from Bethesda Research Laboratories, Bethesda, MD and were used according to the directions of the manufacturer. Digestion was followed to completion by mini-gel analysis. The digested DNAs were subjected to electrophoresis on 0.8% agarose gels in Tris/borate/EDTA buffer (Maniatis et al., 1982). After alkali denaturation and neutralization, the DNA was transferred to nitrocellulose paper (Southern, 1975). After baking the filter at 80°C for 2 h in a vacuum oven, the filter was prehybridized at 42°C in a solution containing 50% formamide, 5× Denhardt's reagent, 5× SSPE (1× SSPE is 150 mM NaCl, 10 mM NaH_2PO_4, 1 mM EDTA), 0.1% SDS, and 100 μg/ml sheared and heat-denatured salmon sperm DNA. Hybridization was carried out at 42°C for 16 h in the above solution containing a ^{32}P-oligolabeled (Feinberg and Vogelstein, 1983, 1984) fibronectin receptor α subunit cDNA consisting of a 675-bp Bam HI fragment from λP7 (Argraves et al., 1986), or ^{32}P-oligolabeled vitronectin receptor α subunit cDNA consisting of a 1,283-bp fragment from λVNR10 (Suzuki et al., 1986).

After hybridization the filters were washed in 2× SSC, 1.5 mM sodium citrate, 0.1% SDS at room temperature for 1 h followed by 1× SSC, 0.1% SDS at 65°C for 1 h. The filters were allowed to air dry and autoradiography was carried out at −70°C using Kodak XAR-5 film and an intensifying screen.

Results

Selection of Cells Resistant to Detachment by the GRGDSP Peptide

When the hexapeptide GRGDSP was added to monolayer cultures of MG-63 cells at a concentration of 0.85 mM, the cells became detached from the substrate as described previously (Hayman et al., 1985). The unattached cells (>99%) were removed and fresh medium containing the peptide (0.85 mM) was added to the remaining cells, some of which also detached from the substrate. These remaining few cells were incubated in the presence of the peptide and eventually a few cells were found to attach and spread. The cells were supplemented with fresh medium containing peptide and were found to be able to attach and grow under these conditions. Once confluent, the cells were detached with EDTA and subcultured in the presence of peptide. The concentration of the hexapeptide was subsequently increased from 0.85 to 5.0 mM in steps of 0.85 mM over a period of 5 mo, each time selecting for attached cells.

To address the question of the stability of the peptide for long periods in culture, spent medium from cells growing in the presence of the peptide for several days was applied to MG-63 cells not previously exposed to the peptide. These cells immediately detached from their culture substrates, indicating that the peptide was still active after several days in culture. Replicate cultures were allowed to grow in the presence of similar concentrations of a variant hexapeptide previously found to be inactive as a cell attachment-promoting agent (Pierschbacher and Ruoslahti, 1984*a*) and ineffective

in detaching cells from their culture substrates (Hayman et al., 1985). This peptide, GRGESP, where E is glutamic acid, did not have any effect on the MG-63 cells, which grew normally and were indistinguishable from MG-63 cells grown in the absence of this peptide.

Physical Characteristics of the Peptide-resistant Variant Cells

The cells capable of attaching and spreading in the presence of 5.0 mM of GRGDSP peptide are hereafter called peptide-resistant variant (PRV)[1] cells. These cells have a markedly different, star-shaped morphology, with multiple processes, some of which are many times longer than the cell bodies (Fig. 1). These morphologic properties are not unlike those of osteocytes (Triffitt, 1980). The MG-63 cells, on the other hand, are essentially flat polygonal cells resembling fibroblasts. The PRV cells appear to interweave and the cell processes are often seen to make connections with one another. In addition, the PRV cells appear to be more secretory as judged by the granular nature of the cytoplasm and processes of these cells. PRV cells grown in the absence of GRGDSP peptide have maintained this morphology for at least 3 mo, indicating that this morphologic alteration is a stable one.

1. *Abbreviations used in this paper*: HLA, human leukocyte antigen; PRV, peptide-resistant variant.

Karyotypic analyses of the two cell lines were carried out to ensure that they are related and also to determine whether any gross chromosomal changes had taken place in the PRV cells as compared with the MG-63 cells. Chromosomes from 5 MG-63 and 16 PRV cells were analyzed. These analyses showed that all of the cells examined from the two cell lines had in common nine distinguishable chromosome markers (Fig. 2) as well as trisomies of several of the chromosomes and the absence of a normal chromosome 9 (not shown). These observations indicate that the PRV cells are indeed derived from the MG-63 cells. No obvious chromosomal changes indicative of gene amplification were observed in the PRV cells.

The PRV cells also have different growth properties than do the MG-63 cells. They proliferate at a much slower rate, requiring at least 15 d to reach confluency as compared with the 4 d required for the MG-63 cells when plated at the same initial density. Moreover, [^{3}H]thymidine uptake studies showed that, whereas the MG-63 cells incorporated 82,397 $\pm$ 13,000 cpm/10^5 cells per 24 h, the PRV cells incorporated only 8,330 $\pm$ 420 cpm/10^5 cells per 24 h. These data represent means of two separate experiments, each experiment representing mean values of three measurements.

Quantitation of Fibronectin and Vitronectin Receptors and Their mRNA in MG-63 and PRV Cells

Because we expected that a qualitative or quantitative alteration in some of the RGD-dependent cell adhesion receptors

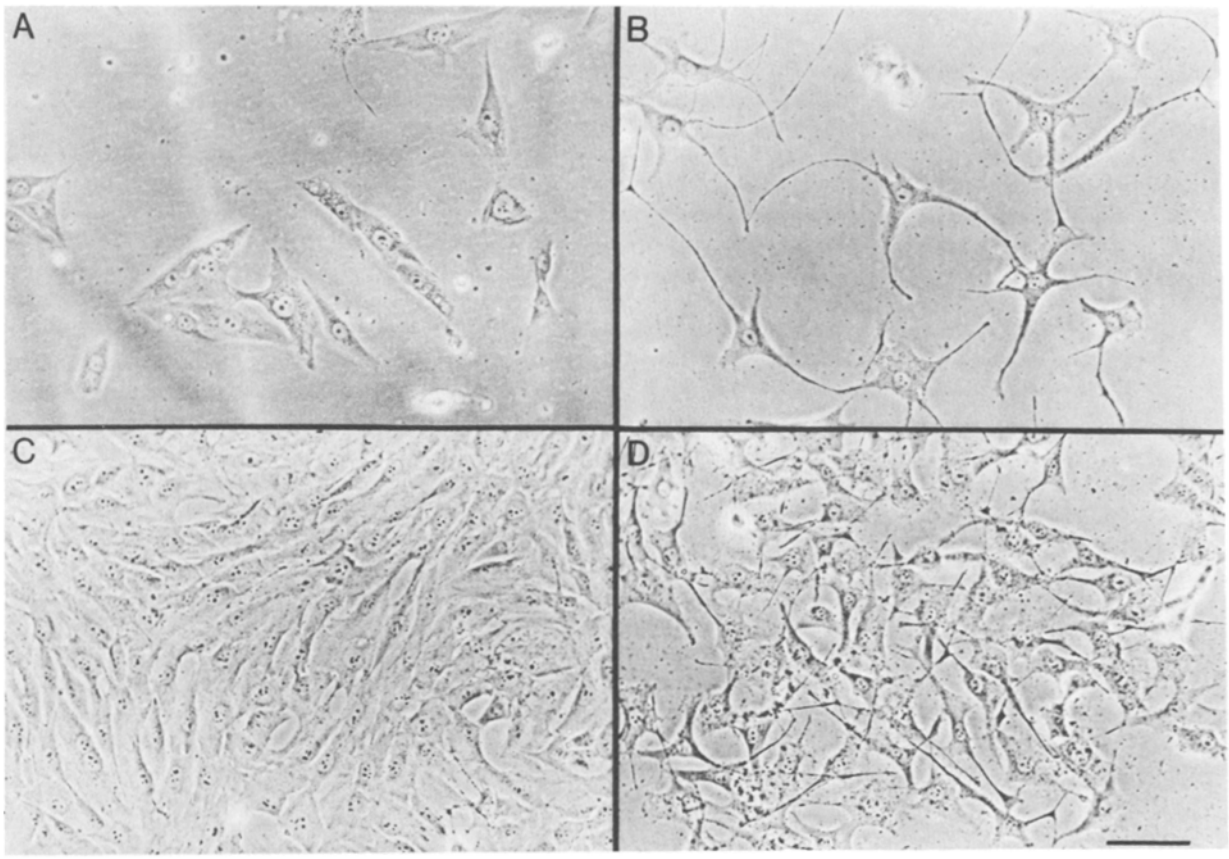

Figure 1. Morphology of MG-63 and PRV cells. Cell monolayers were visualized using a Nikon Diaphot inverted microscope equipped with phase-contrast optics. (*A*) MG-63 cells, sparse culture; (*B*) PRV cells, sparse culture; (*C*) MG-63 cells, dense culture; (*D*) PRV cells, dense culture. Bar, 55 μm.

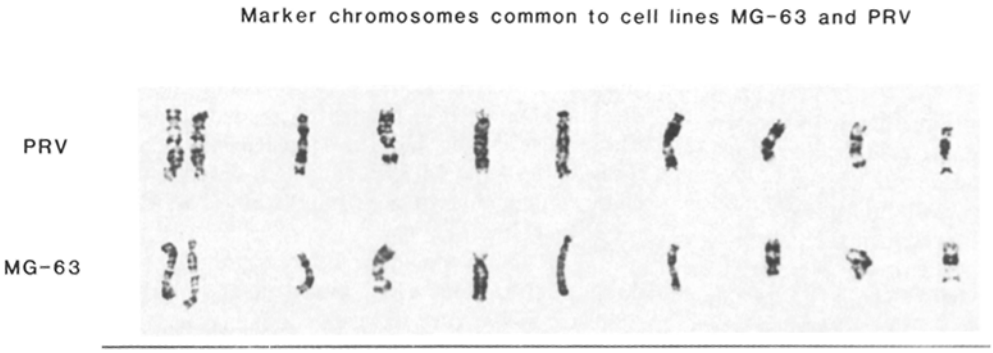

Figure 2. Karyotypic comparison of MG-63 and PRV cells. Marker chromosomes common to both cell lines are shown. The modal number of chromosomes was 63 for MG-63 cells and 57 for PRV cells. Chromosomes from 5 MG-63 and 16 PRV cells were analyzed. Pure trisomies were present in chromosomes 1, 2, 10, 11, 20, and 22 in the MG-63 cells. Trisomie 11 was not present in any of the PRV cells studied while the other trisomies were maintained in all of the cells. Chromosome 9 was lacking in all cells examined.

would account for their ability to attach to substrate in the presence of high concentrations of the RGD-containing peptide, we carried out an analysis of the fibronectin and vitronectin receptors on these cells using affinity-purified polyclonal antibodies reacting with these two receptors. Flow cytometric analysis of the two cell lines indicated that the PRV cells had a significant increase in the mean intensity of fluorescence as compared with the MG-63 cells when the fibronectin receptor was probed in indirect immunofluorescence, whereas no difference in the amount of staining of the vitronectin receptors was observed as compared with the MG-63 cells (Fig. 3). We do not have adequate antibody probes for the collagen receptor (Dedhar et al., 1987) at this time to have allowed us to examine it in this study, but a similar analysis carried out using anti-human leukocyte antigen (HLA) and anti-epidermal growth factor receptor antibodies showed, for comparison, no significant differences in the amounts of these antigens present on the surfaces of these two cell lines (Fig. 3). Moreover, determinations of cell sizes by forward and right-angle light scatter demonstrated that the cells had similar sizes in suspension, indicating that the increased staining with antifibronectin receptor antibodies indeed reflected the presence of increased amounts of receptor on the PRV cells.

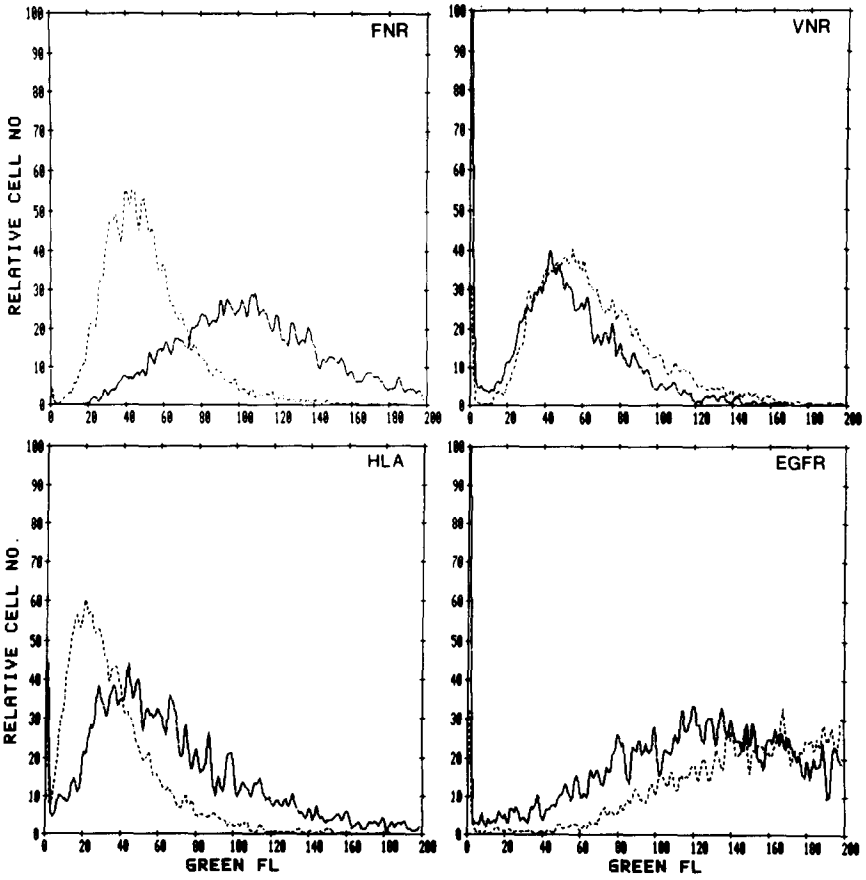

Figure 3. Expression of cell surface proteins on MG-63 and PRV cells. Cells were harvested with 1 mM EDTA and resuspended at a concentration of 2.5×10^5–1×10^6 cells/ml in PBS containing 5% FCS and 0.1% sodium azide. After washing the cells with the above buffer 2 vol (with respect to the cell pellet) of the respective antibody or antiserum was added. After incubation for 1 h at 4°C, the cells were washed several times in the above buffer and FITC-conjugated goat anti-rabbit IgG was added to the cell suspension and incubated for 30 min at 4°C. After extensive washing, the cells were analyzed using a fluorescence-activated cell sorter (Ortho Cytofluorograf 50H). In each case, the broken lines represent the fluorescence profile of MG-63 cells and the solid lines the fluorescence profile of PRV cells. FNR, fibronectin receptor; VNR, vitronectin receptor; EGFR, epidermal growth factor receptor.

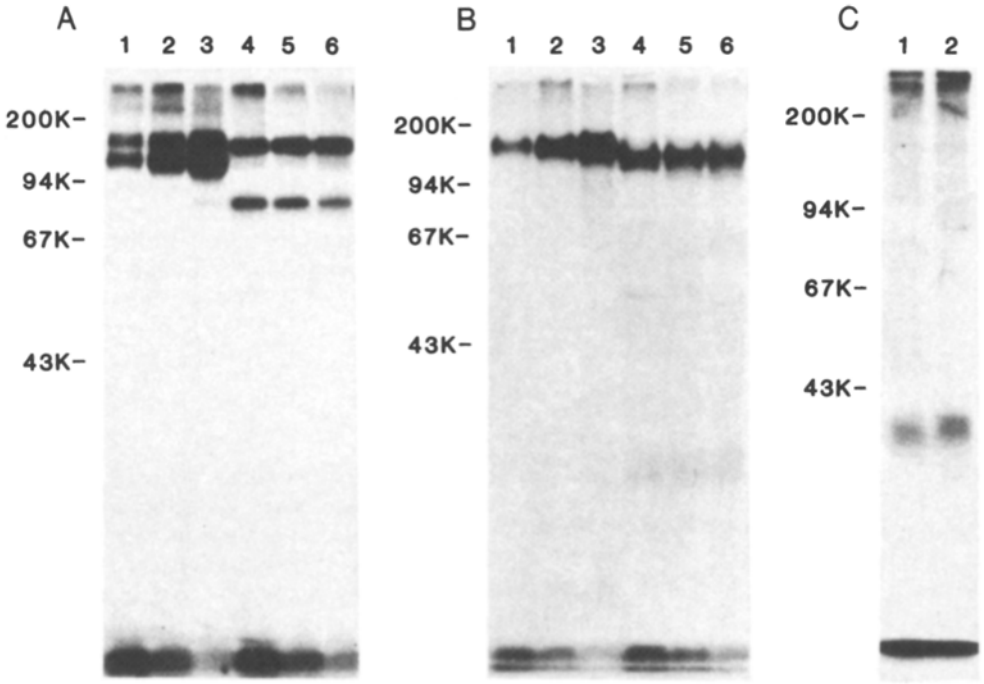

Figure 4. Immunoprecipitates of MG-63 and PRV cell surface antigens recognized by antibodies. MG-63 and PRV cells (~10^7) were surface-labeled with 1 mCi ^{125}I, lysed, and the antigens immunoprecipitated as described in Materials and Methods. Immunoprecipitates were analyzed by SDS-PAGE under nonreducing (*A*) or reducing (*B* and *C*) conditions. (*A* and *B*) Lanes *1–3*, immunoprecipitation with antifibronectin receptor antibody; lanes *4–6*, immunoprecipitation with antivitronectin receptor antibody; lanes *1* and *4*, MG-63 cells (subconfluent culture); lanes *2* and *5*, PRV cells (confluent culture); lanes *3* and *6*, PRV cells (subconfluent culture). (*C*) Immunoprecipitation of subconfluent cultures of MG-63 (lane *1*) and PRV (lane *2*) cells with anti-HLA antibody.

The increase in the number of fibronectin receptors on the PRV cells could also be demonstrated by quantitative immunoprecipitation from ^{125}I surface-labeled cells followed by SDS-PAGE and autoradiography as shown in Fig. 4. The molecular weights of the proteins immunoprecipitated by the respective antibodies agree well with the molecular weights of purified receptors for fibronectin (lanes *1–3*) and vitronectin (lanes *4–6*) and display the characteristic migration patterns on SDS polyacrylamide gels when subjected to electrophoresis under nonreducing and reducing conditions (Pytela et al., 1986). The increase in the number of fibronectin receptors on PRV cells was quantitated by densitometric scanning of the autoradiographs, and the amount of each subunit was found to be approximately sixfold higher than on the parent line of MG-63 cells. A similar analysis of PRV cells growing in the absence of GRGDSP peptide demonstrated that these cells maintained this increase in fibronectin receptor numbers. The number of vitronectin receptors in the PRV cells appears to be similar to that of the MG-63 cells (Fig. 4), consistent with the flow cytometric analysis. Again, for comparison, immunoprecipitation of class 1 HLA antigen with anti-HLA antibodies did not show a significant difference in the amounts of this molecule between the two cell lines (Fig. 4 *C*). These data were consistent in five separate experiments. Furthermore, RNA dot blot analysis of total RNA from PRV and MG-63 cells using ^{32}P-labeled cDNA for the fibronectin receptor α subunit (described in Materials and Methods) indicated that the overproduction of the fibronectin receptor in the PRV cells correlated with a higher level of fibronectin receptor mRNA as compared with the MG-63 cells (Fig. 5). SDS-PAGE of [^{3}H]leucine- and ^{125}I surface-labeled MG-63 and PRV cells failed to reveal any other major differences in the protein profiles of the two cell lines. Taken together, the above data suggest that the PRV cells express more fibronectin receptors than the parental MG-63 cells, and this difference appears to be due to a difference in the amount of mRNA for the fibronectin receptor in the two cell lines.

Southern Blot Analysis

To see whether gene amplification was the cause of the fibronectin receptor overproduction, high molecular weight DNA from MG-63 and PRV cells was digested with restriction endonucleases and analyzed by Southern blot analysis. As can be seen from Fig. 6, the hybridization signal for representative restriction enzyme digests of DNA isolated from both

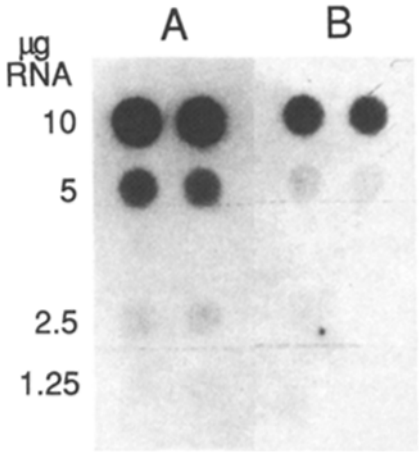

Figure 5. Fibronectin receptor (α subunit) mRNA levels in MG-63 and PRV cells. Cytoplasmic RNA was extracted as described in Materials and Methods, dotted onto nitrocellulose filters, and probed with ^{32}P-labeled cDNA coding for the α subunit of the fibronectin receptor. 10–1.25 μg RNA were applied in duplicate. (*A*) PRV cell RNA; (*B*) MG-63 cell RNA.

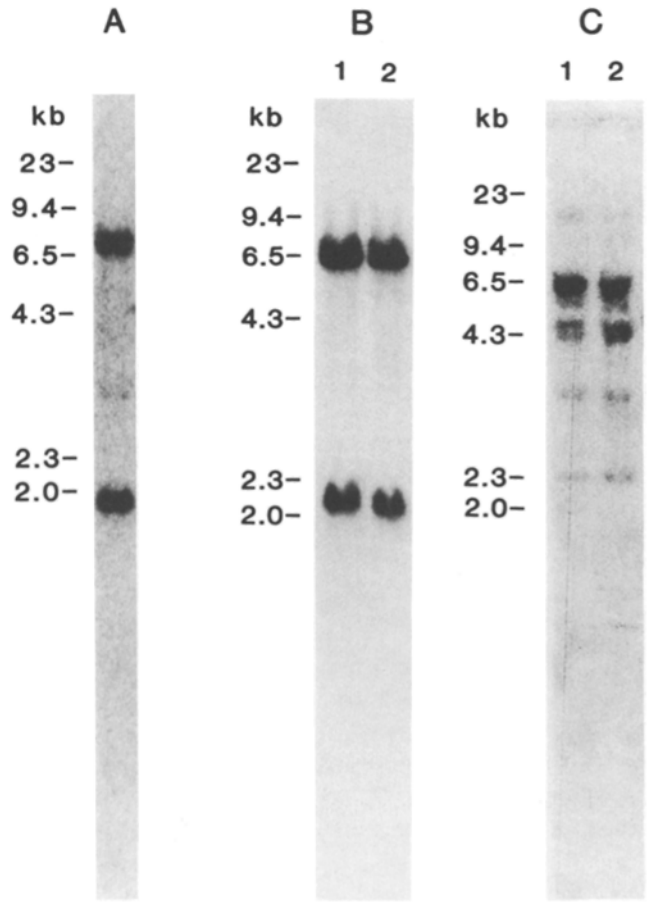

Figure 6. Hybridization of fibronectin and vitronectin receptor cDNA sequences to genomic DNA from MG-63 and PRV cell lines. Restriction enzyme digests of high molecular weight human DNA (20 μg) were separated by electrophoresis on an 0.8% agarose gel, transferred to nitrocellulose filters, and probed with ^{32}P-labeled fibronectin receptor α subunit cDNA (*A* and *B*) or vitronectin receptor α-subunit cDNA (*C*). (A) Human placental DNA; digested with Eco RI. (B) Lane *1*, MG-63 DNA; lane *2*, PRV DNA digested with Eco RI. (C) Lane *1*, MG-63 DNA; lane *2*, PRV DNA digested with Stu I.

cell lines is of equivalent intensity when probed with cDNAs coding for the α subunits of the fibronectin receptor or the vitronectin receptor. The same results were obtained in separate experiments using other restriction enzymes (not shown). It, therefore, appears that the increased expression of the fibronectin receptor in the PRV cells is not due to gene amplification, but is probably due either to an increased rate of transcription of the fibronectin receptor gene or to increased stability of the receptor mRNA.

Ability of MG-63 and PRV Cells to Attach to Fibronectin and Vitronectin in the Presence of GRGDSP Peptide

Unless some qualitative change in the receptors had taken place, one would predict from the increased numbers of fibronectin receptors present on the PRV cells that more peptide would be required to inhibit attachment of these cells to fibronectin than would be required to inhibit attachment of the MG-63 cells to this substrate, whereas similar concentrations of peptide would be required to inhibit attachment of either cell line to vitronectin. This was indeed the case, as is shown in Table I. Approximately 25-fold more peptide was required to achieve 50% inhibition of maximal attachment of PRV cells to fibronectin as compared with the MG-63 cells. However, virtually identical concentrations of peptide were effective at inhibiting attachment of either cell line to vitronectin. These data indicated that the peptide selection process has resulted in cells that adhere more tightly to fibronectin than do the parental cell line and they suggest that, in the presence of the peptide in culture, the PRV cells mainly use the fibronectin receptor, since the amount of peptide present in the selection medium clearly inhibits attachment to vitronectin but is not able to inhibit attachment to fibronectin (Table I).

Discussion

The observation that a synthetic peptide containing the RGD cell attachment-promoting sequence derived from the cell-binding domain of fibronectin (Pierschbacher and Ruoslahti, 1984) can detach cells from culture substrates (Hayman et al., 1985) led us to ask the question whether cells selected for attachment and growth in the presence of this peptide would exhibit alterations in the cell surface receptors for fibronectin or other RGD-containing matrix proteins. We show here that the stepwise selection of MG-63 human osteosarcoma cells for attachment and growth in the presence of cell attachment-inhibiting concentrations of the synthetic peptide, GRGDSP, results in the selection of a cell line capable of growing in the presence of this peptide. These PRV cells overproduce cell surface receptors for fibronectin as compared with the MG-63 cells, and this overproduction is due to an increase in the mRNA levels of the receptor. This increase is, however, not due to the amplification of the fibronectin receptor gene. The PRV cells did not show any differences in the amount of vitronectin receptor, epidermal growth factor receptor, or HLA expressed on their surface. In addition, SDS-PAGE characterization of ^{125}I-labeled total cell surface proteins did not reveal any differences in major cell surface proteins between the two cell lines.

The PRV cells have retained the overproduction of the fibronectin receptor, the resistance of detachment from fibronectin-coated surfaces by the GRGDSP peptide, and the altered morphology for approximately 6 mo, during the last

Table I. Inhibition of Cell Attachment of MG-63 and PRV Cells to Fibronectin- and Vitronectin-coated Surfaces by GRGDSP Peptide

	Concentration of peptide required for 50% inhibition of maximal cell attachment	
Substrate	MG-63	PRV
	mM	*mM*
Fibronectin	0.21	5.30
Vitronectin	0.06	0.07

Assays were carried out in 96-well microtiter plates as described previously (Ruoslahti et al., 1982). 2 μg of fibronectin and 3 μg of vitronectin were coated per well and 10,000 cells were used per well. Maximum cell attachment in the absence of peptide: 60% for fibronectin and 80% for vitronectin.

three of which they were grown in the absence of the GRGDSP peptide. This would indicate that the observed changes are of a genetic rather than an epigenetic origin. However, no gene amplification was observed, and homogeneously staining regions or double minute chromosomes (Stark and Wahl, 1984) were not detected by the chromosome-banding techniques used to examine the chromosomes of the two cell lines. Therefore, it appears that a mechanism exists by which cells can change the amount of fibronectin receptor on their surface and consequently alter their adhesive properties. It is also interesting that both subunits of the fibronectin receptor are overproduced, indicating that the subunits are coordinately expressed and supporting the idea that both subunits are important for interaction with fibronectin (Buck et al., 1986; Santoro and Lawing, 1987).

It is unlikely that we have selected for a very small subpopulation of cells present in the original culture that were resistant to the GRGDSP peptide because culturing MG-63 cells in the presence of 5 mM GRGDSP peptide (the highest concentration used for selection) for extended periods resulted in total cell death. It is more probable that the peptide either induced the up-regulation of the fibronectin receptor or constituted a sustained selective pressure on cells having temporarily increased levels of receptor expression.

One striking consequence of the peptide selection process is the very different morphology of the PRV cells, the most obvious manifestation of which are the long interconnecting processes present on a majority of the PRV cells. This morphology is not unlike that of osteocytes, and it is conceivable that the PRV cells have undergone modulation to a more differentiated phenotype along the osteocyte lineage. Indeed, we have evidence that indicates that the PRV cells are capable of forming a calcified matrix, whereas the MG-63 cells are not (Dedhar, S., and M. Pierschbacher, manuscript in preparation), though a direct cause and effect mechanism cannot be established by this type of study. However, it is entirely possible that the alteration in cell shape may have resulted in the altered phenotypic properties observed in these cells (Watt, 1986; Bissell et al., 1982).

In conclusion, we have shown that it is possible to alter the expression of fibronectin receptor at the surface of at least one cell type in culture by applying an environmental pressure in the form of a synthetic peptide that inhibits cell attachment to fibronectin and vitronectin. The resulting cell line (PRV), which stably overproduces the fibronectin receptor due to an increased level of expression of the fibronectin receptor gene, is more resistant to detachment from fibronectin-coated surfaces than is the MG-63 cell line, grows at a much slower rate, and exhibits a drastically altered morphology compared with the parental MG-63 cells. A further characterization and comparison of these two cell lines should aid in our understanding of the role of extracellular matrix in cell behavior and gene expression, and also in bone cell differentiation.

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