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; Grimell, 1980; Hynes and Yamada, 1982) as well as a variety of changes in cellular behavior (Ali and Hynes, 1978; Pappas 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, 1984a, 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, 1984a; 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., 1985a). 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., 1985b; Dedhar et al., 1987). Moreover, a chicken adhesion receptor interacts with fibronectin and laminin via an RGD-dependent mechanism (Hasagwa 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
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). 32P-sodium iodide, 3H-methylated molecular weight standards, and 32PdCTP 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), tetracycline (10 mg/ml), 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 DNA was hybridized to the human thioredoxin cDNA consisting of a 675-bp Bam HI fragment from ZP7 (Argraves et al., 1986), or a 48-oligolabeled Feinberg and Vogelstein, 1983, 1984) fibronectin receptor α subunit cDNA consisting of a 47-kDa Bam HI fragment from λF7 (Arguave et al., 1986), or a 48-oligolabeled vitronectin receptor α subunit cDNA consisting of a 1.283-bp fragment from XN100 (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 similar concentrations of a variant hexapeptide previously found to be inactive as a cell attachment-promoting agent (Pierschbacher and Ruoslahti, 1984a) 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) 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, [3H]thymidine uptake studies showed that, whereas the MG-63 cells incorporated 82,397 ± 13,000 cpn/10^4 cells per 24 h, the PRV cells incorporated only 8,330 ± 420 cpn/10^4 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

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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.
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 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.

**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 $2.5 \times 10^6$ 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.
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 (Pytel 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 I 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 a 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 $^{35}$S-labeled 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 parent 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
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 32P-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.

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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 32P-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.

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 125I-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
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-bandning 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 the capability of forming a calcified matrix, whereas the MG-63 cells 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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References

Ali, J. V., and R. O. Hynes. 1978. Effects of LETS glycoprotein on cell motility. Cell. 14:439-446.

Argraves, W. S., R. Pytela, S. Suzuki, J. L. Millan, M. D. Pierschbacher, and E. Ruoslabhi. 1986. cDNA sequences from the a subunit of the fibronectin receptor predict a transmembrane domain and a short cytoplasmic peptide. J. Biol. Chem. 261:12922-12924.

Bernard, M. P., J. C. Myers, M. L. Chu, F. Ramirez, E. G. Eikenberry, and D. J. Prockop. 1983. Structure of a cDNA for Pro a 2 chain of type I procollagen. Comparison with chick cDNA for Pro a 2 (I) identifies structurally conserved features of the protein and the gene. Biochemistry. 22:1139-1145.

Billlau, A., G. V. G. Edy, H. Hereman, J. Van Damme, D. Desmyter, J. A. Georgiades, and P. Desomer. 1977. Human interferon: mass production in a newly established cell line, MG-63. Antimicrob. Agents Chemother. 12:11-15.

Bissell, M. J., H. G. Hall, and G. Parry. 1982. How does the extracellular matrix direct gene expression? J. Theor. Biol. 99:31-68.

Blin, N., and D. W. Stafford. 1976. Isolation of high-molecular weight DNA. Nucleic Acids Res. 3:2303-2308.

Buck, C. A., E. Shea, K. Duggan, and A. F. Horwitz. 1986. Integrin (the CSAT antigen): functionality requires oligomeric integrity. J. Cell Biol. 103:2421-2428.

Boucaut, J., T. Darribere, J. T. Poole, H. Aoyama, K. Yamada, and J. P. Thiery. 1984. Biologically active synthetic peptides as probes of embryonic development: a competitive peptide inhibitor of fibronectin function inhibits gastrulation in amphibian embryos and neural crest cell migration in avian embryos. Cell. 99:83-91.

Boucaut, J., E. Ruoslabhi, and M. D. Pierschbacher. 1987. A cell surface receptor complex for collagen type I recognizes the Arg-Gly-Asp sequence. J. Cell Biol. 104:585-593.

Feinberg, A. B., and B. Vogelstein. 1983. A technique for radio labeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. 132:6-13.

Feinberg, A. B., and B. Vogelstein. 1984. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity: addendum. Anal. Biochem. 137:266-287.

Grinnell, F. 1980. Fibroblast receptor for cell-substratum adhesion: studies on the interaction of baby hamster kidney cells with latex beads coated by cold insoluble globulin (plasma fibronectin). J. Cell Biol. 86:104-112.

Hasegawa, T., E. Hasegawa, W.-T. Chen, and K. M. Yamada. 1985. Characterization of a membrane-associated glycoprotein complex implicated in cell adhesion to fibronectin. J. Cell. Biol. 106:307-318.

Hayman, E. G., M. D. Pierschbacher, and E. Ruoslabhi. 1985. Detachment of cells from culture substrate by soluble fibronectin peptides. J. Cell Biol. 100:1948-1954.

Horwitz, A. K., K. Duggan, R. Greggs, C. Decker, and C. Buck. 1985. The cell substrate attachment (CSAT) antigen has properties of a receptor for laminin and fibronectin. J. Cell Biol. 101:2134-2144.

Humphries, M. J., K. Olden, and K. M. Yamada. 1986. A synthetic peptide from fibronectin inhibits experimental metastasis of murine melanoma cells. Science (Wash. DC). 233:467-470.

Hynes, R. O., and K. M. Yamada. 1982. Fibronectins: multifunctional modular glycoproteins. J. Cell Biol. 95:369-377.

Klebe, R. J. 1974. Isolation of a collagen-dependent cell attachment factor. Nature (Lond.). 250:248-251.

Laeremans, U. 1970. Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. Nature (Lond.). 227:680-685.

Lebien, T. E., D. R. Boue, J. C. Bradley, and J. H. Kersey. 1982. Antibody affinity may influence antigenic modulation of the common acute lymphoblastic leukemia antigen in vitro. J. Immunol. 129:2287-2292.

Liotta, L. A. 1986. Tumor invasion and metastases: role of the extracellular matrix. Cancer Res. 46:1-7.

Loring, J., B. Ginellius, and J. A. Weston. 1982. Extracellular matrix materials influence quail neural crest differentiation in vitro. Dev. Biol. 90:165-174.

Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning, a laboratory manual. Cold Spring Harbor Laboratory. Cold Spring Harbor, NY.

Pearlstein, E. 1976. Plasma membrane glycoprotein which mediates adhesion of fibroblasts to collagen. Nature (Lond.). 262:497-500.

Penzpaczer, J. P., J. R. Hassell, K. M. Yamada, and R. M. Pratt. 1979. The influence of an adhesive cell surface protein on chondrogenic expression in vitro. Exp. Cell Res. 121:411-415.

Pierschbacher, M. D., E. G. Hayman, and E. Ruoslabhi. 1983. Synthetic peptides with cell attachment activity of fibronectin. Proc. Natl. Acad. Sci. USA. 80:1224-1227.

Pierschbacher, M. D., and E. Ruoslabhi. 1984a. Cell attachment activity of fibronectin can be duplicated by small synthetic fragment of the molecule. Nature (Lond.). 309:30-33.

Pierschbacher, M. D., and E. Ruoslabhi. 1984b. Variants of the cell recognition site of fibronectin that retain attachment-promoting activity. Proc. Natl. Acad. Sci. USA. 81:5985-5988.

Pytela, R., M. D. Pierschbacher, M. H. Ginsberg, E. F. Plow, and E. Ruoslabhi. 1986. Platelet membrane glycoprotein Ib/IIa: member of a family of
arg-gly-asp-specific adhesion receptors. *Science (Wash. DC).* 231:1559–1562.

Pytela, R., M. D. Pierschbacher, and E. Ruoslahti. 1985a. Identification and isolation of a 140 kDa cell surface glycoprotein with properties of a fibronectin receptor. *Cell.* 40:191–198.

Pytela, R., M. D. Pierschbacher, and E. Ruoslahti. 1985b. A 125/115 kDa cell surface receptor specific for vitronectin interacts with the arginine-glycine-aspartic acid adhesion sequence derived from fibronectin. *Proc. Natl. Acad. Sci. USA.* 82:5766–5770.

Ruoslahti, E. 1984. Cellular adhesion, invasion and metastasis. *Biochim. Biophys. Acta.* 738:263–284.

Rubin, K., S. Johansson, I. Pettersson, C. Ocklind, B. Obrink, and M. Hook. 1979. Attachment of rat hepatocytes to collagen and fibronectin: a study using antibodies directed against cell surface components. *Biochem. Biophys. Res. Commun.* 91:86–94.

Ruoslahti, E. 1984. Fibronectin in cell adhesion and invasion. *Cancer Metast. Rev.* 3:43–51.

Ruoslahti, E., and E. G. Hayman. 1979. Two active sites with different characteristics in fibronectin. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 97:221–224.

Ruoslahti, E., E. G. Hayman, and M. D. Pierschbacher. 1985. Extracellular matrices and cell adhesion. *Atherosclerosis.* 5:581–594.

Ruoslahti, E., E. G. Hayman, M. Pierschbacher, and E. Engvall. 1982. Fibronectin: purification, immunochemical properties, and biological activities. *Methods Enzymol.* 82:803–831.

Santoro, S., and W. J. Lawing, Jr. 1987. Competition for related but nonidentical binding sites on the glycoprotein IIb-IIIa complex by peptides derived from platelet adhesion proteins. *Cell.* 48:867–873.

Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* 98:503–517.

Stark, G. R., and G. M. Wahl. 1984. Gene amplification. *Annu. Rev. Biochem.* 53:467–491.

Suzuki, S., W. S. Argraves, R. Pytela, H. Arai, T. Krusius, M. D. Pierschbacher, and E. Ruoslahti. 1986. cDNA and amino acid sequences of the cell adhesion receptor recognizing vitronectin reveal a transmembrane domain and homology with other adhesion receptors. *Proc. Natl. Acad. Sci. USA.* 83:8614–8618.

Suzuki, S., A. Oldberg, E. G. Hayman, M. D. Pierschbacher, and E. Ruoslahti. 1985. Complete amino acid sequence of human vitronectin deduced from cDNA. Similarity of cell attachment sites in vitronectin and fibronectin. *EMBO (Eur. Mol. Biol. Organ.) J.* 4:2519–2524.

Triffitt, J. T. 1980. The organic matrix of bone tissue. In *Fundamental and Clinical Bone Physiology.* M. R. Urist, editor. J. B. Lippincott Co., Philadelphia. 45–82.

Ullrich, A. P., J. Shine, J. Chirgwin, R. Pictet, E. Tischer, W. J. Rutter, and H. M. Goodman. 1977. Rat insulin genes: construction of plasmids containing the coding sequences. *Science (Wash. DC).* 196:1313–1315.

Watt, F. M. 1986. The extracellular matrix and cell shape. *Trends Biol. Sci.* 11:482–485.

Yamada, K. M. 1983. Cell surface interactions with extracellular materials. *Annu. Rev. Biochem.* 52:761–790.

Yamada, K. M., and D. W. Kennedy. 1984. Dualistic nature of adhesive protein function: fibronectin and its biologically active peptide fragments can autoinhibit fibronectin function. *J. Cell Biol.* 99:29–36.