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ANTIBODY AGAINST HUMAN α1β1 INTEGRIN INHIBITS HELA CELL ADHESION TO LAMININ AND TO TYPE I, IV, AND V COLLAGENS

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Summary : In HeLa cells β1 integrin forms heterodimers with α1, α2, α3, α5 and α6 integrin subunits. Integrin αvβ5 can also be detected. A monoclonal antibody SR-84 identified the α1 integrin subunit in immunoprecipitation assays and inhibited α1-related cell adhesion to different matrix proteins, laminin-1 and type I, IV, and V collagens, whereas its effect on adhesion to type II collagen was marginal. HeLa cells do not attach to type VI collagen. The presence of magnesium was essential for HeLa cell adhesion, whereas calcium alone was not sufficient and high concentrations of calcium even counteracted the effect of magnesium. Cell adhesion to type I collagen was sensitive to changes in pH, unlike cell adhesion to type IV collagen. We conclude that SR-84 is a valuable tool to study α1 integrin-related functions, and that in HeLa cells α1β1 integrin is a magnesium-dependent receptor for type I, IV, and V collagens but not for type II and VI collagens.

Integrins are a large family of integral membrane proteins forming heterodimeric receptors for various ligands, including extracellular matrix molecules [1,2]. Integrin-type receptors are composed of two subunits, α and β. Fifteen α chains, eight β chains and 21 different α/β complexes are known to date. The diversity of integrins is further increased by alternative splicing. Heterodimers containing either β1 or αv subunit are usually receptors for extracellular matrix molecules, including collagens, laminins, and fibronectin. A single cell can express simultaneously many integrin-type receptors for one matrix molecule and one integrin heterodimer can have multiple ligands. Intracellular domain of β1 integrin is connected to cytoskeleton, whereas the intracellular domains of α chains are suggested to be responsible for the signal transduction function of integrins. Integrin-mediated signals regulate cell phenotype, differentiation, migration, and gene expression [3].

The α1β1 integrin is also known as very late activation antigen-1 (VLA-1), since inflammatory cells start to express the molecule only several days after *in vitro* stimulation [4]. *In vivo* α1β1 integrin expression is seen in synovial lymphocytes of patients with rheumatoid arthritis [5].

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Several cytokines regulate the expression of α1 integrin subunit in mesenchymal cells, for example transforming growth factor-β (TGF-β), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α) increase α1 synthesis in fibroblasts [6,7]. In osteogenic MG-63 cells, which do not normally express α1β1 integrin IL-1β can induce its expression [7]. In PC-12 cells, nerve growth factor (NGF) induced α1 expression, increased adhesion to laminin and differentiation toward neuronal cells [8]. In fibroblast culture, cell quiescence increases α1 integrin expression [9].

Rat [10] and human α1 integrin cDNAs [11] have been isolated and sequenced. Human α1 integrin contains a 206 amino acid I-domain, typical for collagen binding integrins. Ligand binding specificity and affinity of different integrins, including α1β1, has been tested by ligand affinity chromatography and by binding assays with isolated integrins [12-17]. In solid-phase assays α1β1 integrin interacts at least with laminin-1, type I, III, IV, and VI collagens. However, isolated integrins may not have the same ligand binding specificities than the corresponding molecules expressed on cell surfaces. Since a function-blocking anti-human α1 integrin antibody has not been generally available for these studies, less is known about the ligand binding specificity of human α1β1 integrin when it is expressed on intact cells.

Materials and Methods

Antibodies. Polyclonal rabbit antisera against human β1 [6], α2 and α3 integrin subunits [18], and monoclonal antibodies against α5 [19], α6 [20], αv [21], β3 [22], and β5 [23] integrins were used in immunoprecipitation assays. Monoclonal antibodies specific to α1 integrin subunit were SR-84 [24] and TS2/7 [25]. SR-84 IgG was purified from rat ascites on a protein G-sepharose (Pharmacia) column. IgG was eluted from the column with 0.9% NaCl and 0.58% acetic acid. The eluted solution was dialysed in 50mM NH_4HCO_3 (pH 7.4) at 4°C for 12 h, frozen to -70°C and lyophilised. Purified IgG was dissolved in phosphate-buffered saline (pH 7.4). The purity and concentration of IgG were estimated by SDS-PAGE and silver-staining.

Immunoprecipitations. Cell cultures were metabolically labelled with 50 μCi/ml of a mixture of [^{35}S]methionine and [^{35}S]cysteine (Tran^{35}S-label, ICN Biochemicals) in methionine-free minimum essential medium for 24 h. Integrins were immunoprecipitated from octylglycoside-extracts with specific antibodies as described [18]. Immunoprecipitates were analyzed by electrophoresis on 6% sodium dodecyl sulfate polyacrylamide gels under nonreducing conditions followed by fluorography. Integrin bands were quantified from fluorograms by the Microcomputer Imaging Device version M4 (Imaging Research Inc).

Cell adhesion assays. The coating of a 96-well immunoplate (Maxi Sorp, Nunc, Denmark) was done by exposure to 0.2 ml of phosphate-buffered saline (pH 7.4) containing 4-5 μg/cm^2 laminin-1 (purified from basement membranes of the Engelbreth-Holm-Swarm, EHS, mouse tumor, Collaborative Research), fibronectin (human plasma fibronectin, Boehringer Mannheim), type I collagen (from lathyric rat skin, Boehringer Mannheim), type II collagen (from chicken sternal cartilage, Genzyme, Boston, or from bovine cartilage, a kind gift from Dr. Michel van der Rest, Lyon, France), type IV collagen (Pepsin extracted from human placenta, Sigma, or native type IV collagen from mouse EHS tumors, Sigma), type V collagen (human, Chemicon) or type VI collagen (human, Chemicon) at 4°C for 12 h. Bovine serum albumin was used to measure the non-specific binding. Residual protein absorption sites in all wells were blocked with 1 % bovine serum albumin in phosphate-buffered saline for 1 h at 37°C. Confluent cell cultures were detached by using 0.01% trypsin and 0.02% EDTA. Trypsin activity was inhibited by washing the cells with 1 mg/ml of soybean trypsin inhibitor (Sigma). Cells were suspended in DMEM (Life Technologies, Inc.), HEPES buffer (pH 6.0-7.5; 1 mM $MgCl_2$) or HANKS solution (pH 7.4; 0–5 mM $CaCl_2$ or $MgCl_2$). 10,000 cells were transferred into each well and incubated at 37°C for 45 min. Non-adherent cells were removed by rinsing the wells with medium. Adherent cells were fixed with 2 % paraformaldehyde, stained with 0.5 % crystal violet in 20% ethanol and washed with distilled water. The immunoplates were allowed to air-dry, and the stained cells were dissolved into 10 % acetic acid and spectrophotometrically measured at 600 nm with Multiscan Plus (Lab-Systems).

Results and Discussion

HeLa cells were metabolically steady-state labelled with [^{35}S]methionine and integrins were immunoprecipitated with specific antibodies. Anti-β1 integrin antiserum immunoprecipitated three major protein bands with relative molecular mass of 130, 150, and 190 kDa (Fig. 1A). These represented β1 integrin, multiple β1-associated 150 kDa α subunits, and α1 subunit, respectively. The exact identities of the 150 kDa α subunits were studied by using specific monoclonal antibodies and antisera. HeLa cells expressed α2, α3, α5, and α6 subunits in complex with β1 integrin subunit (Fig. 1B). The identity of the 190 kDa band was confirmed by using previously described anti-α1 integrin antibodies, TS2/7 and SR-84 (Fig. 1A). Quantitative analysis of β1-associated α subunit bands in immunoprecipitation assays showed that α1β1 comprice 60-70 % of β1 integrins in HeLa cells. We could also show the presence of αvβ5, whereas no αvβ3 was detected (Fig. 1B). Thus, HeLa cells were considered as an exellent tool to study the specificities and functions of the human cellular α1β1 integrin heterodimer.

Monoclonal antibody SR-84 is specific for the human α1 integrin subunit. We show here that unlike many other anti-human α1 antibodies, including TS2/7, SR-84 can also inhibit the adhesion of cells to matrix molecules. SR-84 could completely block the adhesion of HeLa cells to type IV collagen (Figs. 2A, 2B, and 3). Maximal effect was already seen in concentration as low as 1

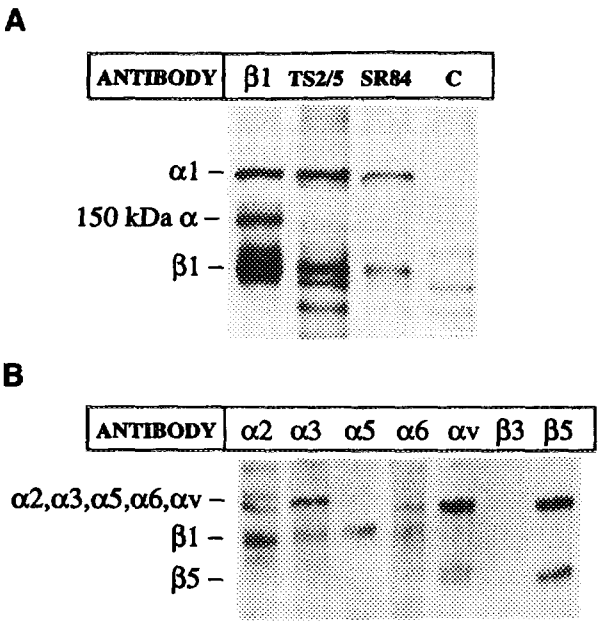

Fig.1. Expression of integrins in HeLa cells. Confluent cultures of HeLa cells were metabolically steady state labelled with [^{35}S]methionine in methionine-free medium. Cells were harvested and aliquots of detergent soluble cell extracts were immunoprecipitated with antibodies against β1 and α1 (SR-84 and TS2/5) (A) or α2, α3, α5, α6, αv, β3, and β5 (B) integrin subunits. Immunoprecipitants were analysed by SDS-PAGE under non-reducing conditions followed by fluorography.

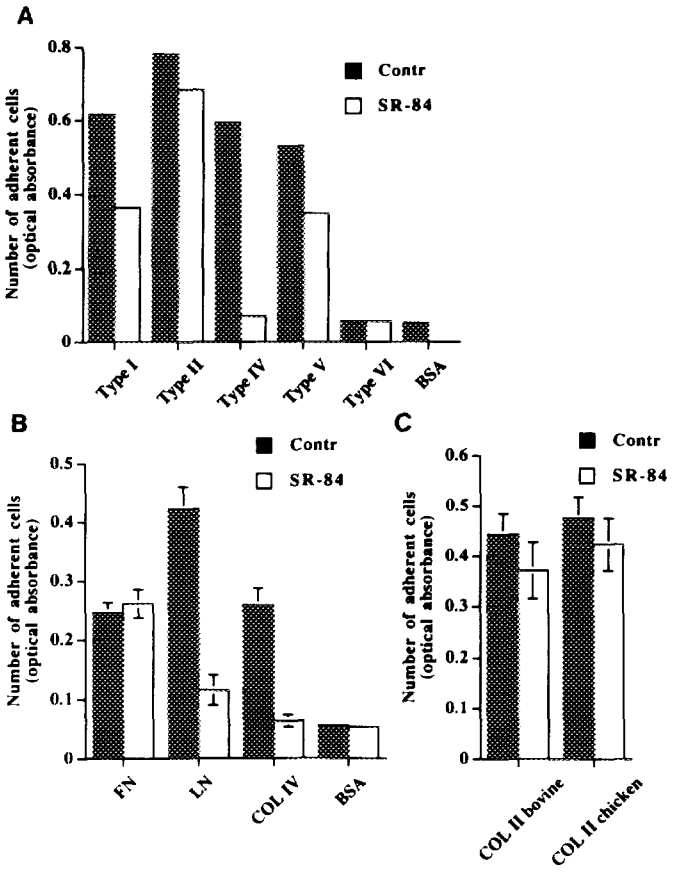

Fig.2. The effects of monoclonal antibody SR-84 on the adhesion of HeLa cells to different substrata. 96-well plates were incubated with 0.2 ml of phosphate-buffered saline (pH 7.4) containing 4-5 μg/cm^2 of the appropriate matrix component. Residual protein absorption sites were blocked with bovine serum albumin and it was also used to measure the nonspecific binding. Cells were trypsinized, washed with medium containing soybean trypsin inhibitor and suspended in DMEM. 10,000 cells were transferred to each well. After 45 min of incubation at 37°C, wells were rinsed and adherent cells were fixed, stained, dissolved in acetic acid and measured spectrophotometrically.

μg/ml (Fig. 3). In the presence of SR-84 cell adhesion to type I collagen was constantly only slightly higher than to BSA (Figs. 2A and 3). SR-84 could partially inhibit cell adhesion to laminin and to type V collagen (Figs. 2A and B). However, the protein concentration needed was higher than in the blocking of adhesion to type I or type IV collagens (Fig. 3). SR-84 could not inhibit adhesion to fibronectin and its effect on the adhesion of HeLa cells to type II collagen was marginal (Figs. 2A, 2B, and 2C). HeLa cells could not attach to type VI collagen (Fig. 2A), suggesting that α1β1 integrin is not a receptor for it. HeLa cell adhesion to type II collagen was studied by using two different preparations of collagen, one from chicken and another from bovine cartilage, with identical results (Fig. 2C). Cell adhesion to type IV collagen was dependent on whether native type IV collagen or a preparation purified by pepsin extraction was used, since SR-84 could only partially inhibit the adhesion of HeLa cells to the latter (not shown).

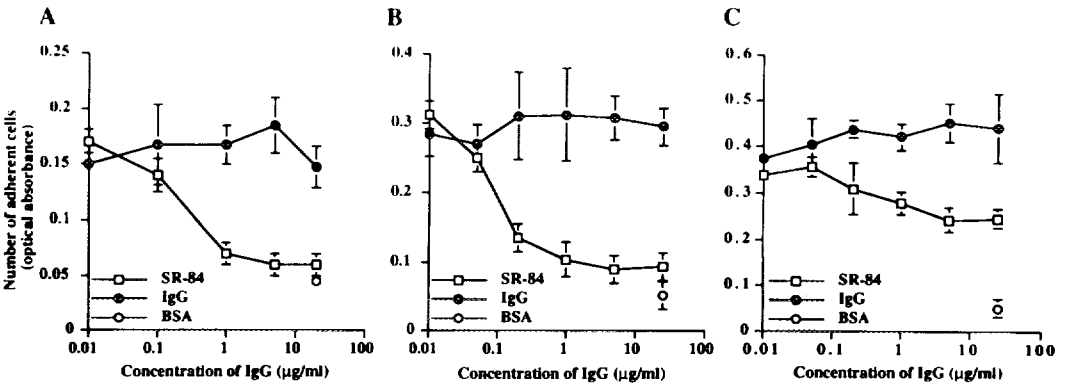

Fig.3. The adhesion of HeLa cells to type I and IV collagens and to laminin-1 using different concentrations of the antibody SR-84. 96-well plates were coated with type IV collagen (A), type I collagen (B), or laminin (C). After treatment with medium containing soybean trypsin inhibitor, 10,000 trypsinized cells were suspended in DMEM containing SR-84 or control rat IgG and transferred into wells. After 45 min of incubation at 37°C, wells were rinsed and adherent cells were fixed, stained, dissolved in acetic acid and measured spectrophotometrically.

Collagens represent a large family of proteins that contribute to the extracellular matrix of many tissues [26]. To date 19 different types of collagens are known, namely collagens I-XIX. These trimeric molecules can be divided into fibrillar collagens and collagens with interrupted helices. Fibrillar collagens (types I, II, III, V and XI) have a continous triple helix, which is about 300 nm long, whereas 14 different types of non-fibrillar collagens comprise of non-continuous triple-helices. One major aim of this study was to analyze the binding specificity of α1β1 integrin in detail. Three fibrillar (types I, II, and V), and two non-fibrillar (types IV and VI) collagens were available for analysis. In HeLa cells α1β1 integrin was clearly found to be a receptor for type I, IV and V collagens. However, additional receptors may be involved in HeLa cell adhesion to type I and V collagen. By contrast, α1β1 does not seem to interact with type II and IV collagens, suggesting that the interaction of α1β1 integrin with collagens is not dependent on whether the collagen has a fibrillar or non-fibrillar structure. Members of both collagen groups contain ligands for α1β1, and both groups seem to have collagen types not interacting with α1β1 integrin.

Our results are in accordance with several other reports indicating that α1β1 can mediate cell adhesion to laminin-1 (previously called EHS laminin) but not to fibronectin. In HeLa cells SR-84 could block adhesion to laminin-1 only partially, suggesting the presence of an alternative laminin receptor. The fact that SR-84 blocks the function of α1β1 is also shown in a recent report by Choi et al. [27], in which SR-84 inhibited the adhesion of neuronal SY5Y cells to laminin. Two functional anti-rat α1 integrin antibodies, mAb 3A3 [28] and mAb 33.4 [29] have been described, but only 3A3 has been reported to block cell adhesion to laminin [28].

Since α1β1 is the major integrin in HeLa cells, and SR-84 could almost completely prevent the adhesion of HeLa cells to type I and IV collagens, the interaction of α1β1 integrin with these substrates in HeLa cells can be studied without interference of other integrin-type collagen receptors. In the presence of Ca^{++} (from 0.1 to 5 mM) there was no HeLa cell adhesion to either

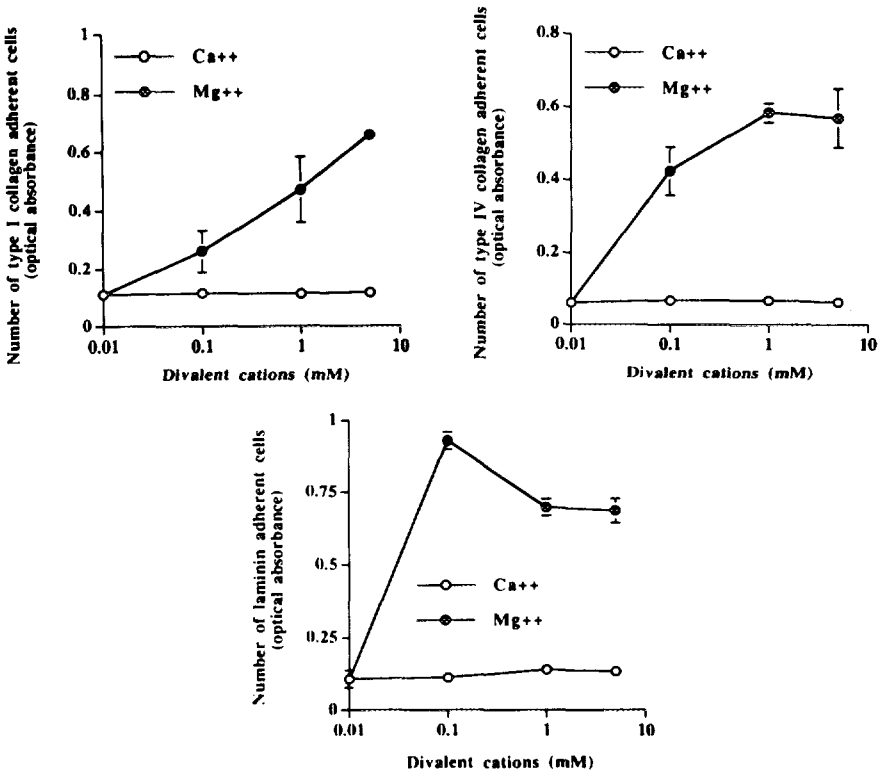

Fig.4. The effects of various concentrations of Mg^{++} and Ca^{++} on the adhesion of HeLa cells to type I and IV collagens and to laminin-1. 96-well plates were coated with type IV collagen, type I collagen or laminin-1. After treatment with medium containing soybean trypsin inhibitor, 10,000 trypsinized cells were suspended in HANKS solution containing various concentrations of Mg^{++} and Ca^{++} and transferred into wells. After 45 min of incubation at 37°C, wells were rinsed and adherent cells were fixed, stained, dissolved in acetic acid and measured spectrophotometrically.

type I or to type IV collagen (Fig. 4). However, in relatively small Mg^{++} concentrations (0.5–1.0 mM) a maximal adhesion to type IV collagen was detected (Fig. 4). Maximal adhesion to type I collagen required larger (5 mM) concentrations of Mg^{++} (Fig. 4). The adhesion of HeLa cells to laminin was also dependent on the presence of Mg^{++} (Fig. 4). Furthermore, in the presence of 1 mM Mg^{++}, high Ca^{++} concentrations (5 mM) could inhibit cell adhesion to collagens types I and IV (Fig. 5). Similar phenomenon has been described for $\alpha 2\beta 1$ integrin [30]. This inhibition was not seen with cells adhering to laminin-1 (not shown). The adhesion of HeLa cells to type I collagen was sensitive to changes in pH (Fig. 6). However, cell adhesion to type IV collagen and laminin was much more resistant to slightly acidic pH (pH 6.0–7.0; Fig. 6). Our results indicate that even if $\alpha 1\beta 1$ integrin is the major receptor for both type I and IV collagens in HeLa cells, the physico-chemical environments needed for optimal interaction between $\alpha 1\beta 1$ integrin and these two ligands may differ. The molecular details responsible for these differences between HeLa cell – type I collagen and HeLa cell – type IV collagen interactions remain to be determined.

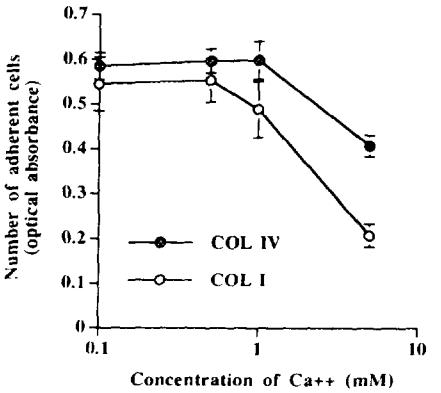

Fig.5. The inhibition of HeLa cell adhesion to type I and IV collagens by various concentrations of Ca++. After treatment with medium containing soybean trypsin inhibitor, 10,000 trypsinized cells were suspended in HANKS solution (pH 7.5, 1 mM $MgCl_2$) containing various concentrations of Ca++ and transferred into wells. After 45 min of incubation at 37°C, wells were rinsed and adherent cells were fixed, stained, dissolved in acetic acid and measured spectrophotometrically.

Integrin α1β1 is an important component in the apparatus mediating cellular interaction with different collagenous components of extracellular matrix. Our data show that α1β1 can discriminate between different types of collagens and regulate the ability of cells to adhere to their surroundings. It is probable that, like other integrins, also α1β1 integrin can mediate signals into cells. It is thus tempting to speculate that, respectively, different collagen types might generate different cellular responses.

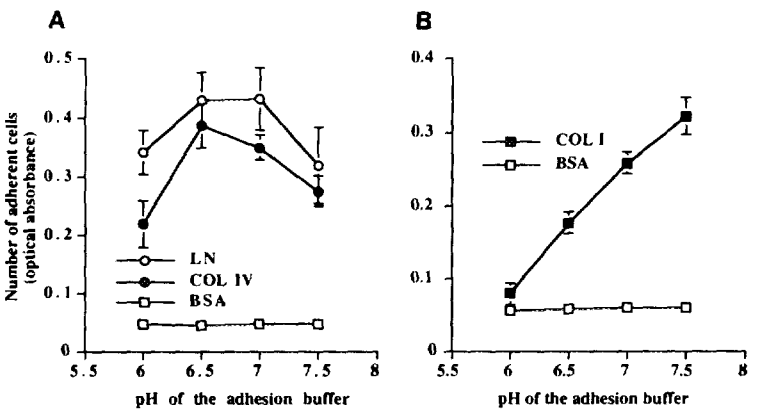

Fig.6. The effect of pH of the adhesion buffer on the ability of Hela cells to attach to type I and IV collagens. After treatment with medium containing soybean trypsin inhibitor, 10,000 trypsinized cells were suspended in HEPES buffer (pH 6, 6.5, 7, or 7.5) containing 1mM $MgCl_2$ and transferred into wells. After 45 min of incubation at 37°C, wells were rinsed and adherent cells were fixed, stained, dissolved in acetic acid and measured spectrophotometrically.

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References

1 Hynes, R. O. (1992) Cell 69, 11-25
2 Ruoslahti, E. (1991) J. Clin. Invest. 87, 1-5
3 Schwartz, M.A. (1992) Trends Cell Biol. 2, 304-308
4 Hemler, M.E., Jacobson, J.G. and Strominger, J.L. (1985) J. Biol. Chem. 260, 15246-15252
5 Hemler, M.E., Glass, D., Coblyn, J.S. and Jacobson, J.G. (1986) J. Clin. Invest. 78, 696-702
6 Heino, J., Ignotz, R. A., Hemler, M. E., Crouse, C. and Massagué, J. (1989) J. Biol. Chem. 264, 380-388
7 Santala, P. and Heino, J. (1991) J. Biol. Chem. 266, 23505-23509
8 Tomaselli, K.J., Damsky, C.H. and Reichardt, L.F. (1987) J. Cell Biol. 105, 2347-2358
9 Fingerman, E. and Hemler, M.E. (1988) Exp. Cell Res. 177, 132-142
10 Ignatius, M.J., Large, T.H., Houde, M., Tawil, J.W., Barton, A., Esch, F., Carbonetto, S. and Reichardt, L.F. (1988) J. Cell Biol. 111, 709-720
11 Briesewitz, R., Epstein, M.R. and Marcantonio, E.E. (1993) J. Biol. Chem. 268, 2989-2996
12 Kramer, R.H. and Marks, N. (1989) J. Biol. Chem. 264, 4684-4688
13 Gullberg, D., Terracio, L., Borg, T.K. and Rubin, K. (1989) J. Biol. Chem. 264, 12686-12694
14 Kern, A., Eble, J., Golbik, R. and Kühn, K. (1993) Eur. J. Biochem. 215, 151-159
15 Eble, J.A., Golbik, R., Mann, K. and Kühn, K. (1993) EMBO J. 12, 4795-4802
16 Gullberg, D., Gehlsen, K.R., Turner, D.C., Ahlén, K., Zijenah, L.S., Barnes, M.J. and Rubin, K. (1992) EMBO J. 11, 3865-3873
17 Pfaff, M., Aumailley, M., Specks, U., Knolle, J., Zerwes, H.G. and Timpl, R. (1993) Exp. Cell Res. 206, 167-176
18 Santala, P., Larjava, H., Nissinen, L., Riikonen, T., Määttä, A. and Heino, J. (1994) J. Biol. Chem. 269, 1276-1283
19 Werb, Z., Tremble, P.M., Behrendtsen, O., Crowley, E. and Damsky, C.H. (1989) J. Cell Biol. 109, 877-889
20 Sonnenberg, A., Hogervorst, F., Osterop, A. and Veltman, F.E.M. (1988) J. Biol. Chem. 263, 14030-14038
21 Wayner, E.A., Orlando, R.A. and Cheresh, D.A. (1991) J. Cell Biol. 113, 919-929
22 Ylänne, J., Hormia, M., Järvinen, M., Vartio, T. and Virtanen, I. (1988) Blood 72, 1478-1486
23 Weinacker, A., Chen, A., Agrez, M., Cone, R.I., Nishimura, S., Wayner, E., Pytela, R. and Sheppard, D. (1994) J. Biol. Chem. 269, 6940-6948
24 Rettig, W.J., Dracopoli, N.C., Goetzger, T.A., Spengler, B.A., Biedler, J.L., Oettgen, H.F. and Old, L.J. (1984) Proc. Natl. Acad. Sci. USA 81, 6437-6441
25 Hemler, M.E., Sanchez-Madrid, F., Flotte, T.J., Krensky, A.M., Burakoff, S.J., Bhan, A.K., Springer, T.A. and Strominger, J.L. (1984) J. Immunol. 132, 3011-3018
26 Kivirikko, K. (1993) Ann. Med. 25, 113-126
27 Choi, E.S.-H., Rettig, W.J., Wayner, E.A., Srour, M.L. and Clegg D.O. (1994) J. Neurosci. Res. 37, 475-488
28 Turner, D.C., Flier, L.A. and Carbonetto, S. (1989) J. Neurosci. (1989) 9, 3287-3296
29 Löster, K., Voigt, S.V., Heidrich, C., Hofmann, W. and Reutter, W. (1994) Exp. Cell Res. 212, 155-160
30 Grzesiak, J.J., Davis, G.E., Kirchhofer, D. and Pierschbacher, M.D. (1992) J. Cell Biol. 117, 1109-1117