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A Role of Fibronectin in the Extracellular Matrix during Chick Myoblast Differentiation

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Our previous report has suggested that the decrease of fibronectin level during myogenesis is due to the decreased availability of receptor (matrix assembly receptor) for 29-kDa fragment of fibronectin. In the present study, we demonstrate that G protein and adenylate cyclase system are involved in the regulation of fibronectin matrix assembly and that when fibronectin level in extracellular matrix decreases, the postmitotic fusion-capable cells emerge more frequently from the proliferative population. This proposal is based on the following observations. (1) Cholera toxin, which increases intracellular cAMP, caused a decrease in the ability of myoblasts to incorporate fibronectin into extracellular matrix. (2) Cholera toxin decreased the proliferation of myoblasts and induced the precocious fusion. (3) dbcAMP, which was found to induce the precocious fusion and decrease the proliferation of myoblasts, decreased the fibronectin level in extracellular matrix and matrix assembly receptor for fibronectin. (4) RGDS, which inhibits the incorporation of fibronectin into extracellular matrix, induced the precocious fusion and reduced the proliferaton of myoblasts. These results suggest that cAMP regulates the fibronectin levels in extracellular matrix and that the alteration of fibronectin level is involved in regulation of the proliferation and differentiation of chick embryonic myoblasts.

KEY WORDS: Myoblast Differentiation, Fibronectin, Cholera Toxin, 29-kDa Fragment

Myoblasts, the cycling precursor cells for vertebrate skeletal muscle, are capable of undergoing differentiation in cell culture into postmitotic, fusion-competent, contractile muscle cells (Knudsen and Horwitz, 1977). Skeletal muscle fibers are surrounded by an extracellular matrix composed of collagen, fibronectin, laminin, and proteoglycan (Olwin and Hall, 1985). Because the extracellular matrix is an important determinant of cell growth, migration, and differentiation, cellular regulation of fibronectin matrix formation, as well as regulation of fibronectin synthesis, could serve as an important clue of myoblast differentiation. Several lines of evidence suggest that fibronectin is a key molecule of myoblast differentiation as follows: 1) Addition of fibronectin to myoblasts inhibits the myoblast fusion and removal of fibronectin from cell surface with trypsin induces the precocious fusion (Podlesky *et al.*, 1979). 2) Immunocytochemical observation on the L6 muscle cell line and cloned human muscle cells indicates that as myoblasts fuse they lose their surface fibronectin (Walsh *et al.*, 1981). 3) Similiar results are obtained using chick embryonic myoblasts as well (Chung and Kang, 1987).

The decrease of fibronectin during myogenesis is due to the decrease of fibronectin matrix assembly site but not to the decrease of cellular synthesis of fibronectin (Gardner and Fambrough 1983; Chung and Kang, 1990). The assembly of a fibronectin matrix is a complex and tightly regulated process which involves interaction between integrin and matrix assembly receptor. Integrin binds to fibronectin with modest affinity via the recognition sequence RGD (Arg-Gly-Asp) in the type III homology unit in the cell adhesion domain of fibronectin (Pierschbacher and Ruoslahti, 1984; Akiyama *et al.*, 1989). Matrix assembly receptor binds to fibronectin more tightly than the integrin and shows specificity for the disulfide-bonded type I homology units of fibronectins rather than the cell adhesion domain (McKeown-Longo and Mosher, 1985; McDonald *et al.*, 1987; Chung and Kang, 1990).

Various compounds have been shown to modulate fibronectin binding and matrix assembly. Serum enhances fibronectin binding and assembly in mouse fibroblasts (Bowlus *et al.*, 1991). Hormones and growth factors have been shown to modify extracellular matrix assembly. Adenylate cyclase systems are involved in the regulation of fibronectin matrix assembly in fibroblasts (Allen-Hoffmann and Mosher, 1987). In this report, we examined if cholera toxin, which increases intracellular cAMP, causes a decrease in the ability of myoblasts to incorporate fibronectin into extracellular matrix and how fibronectin regulates the myoblast differentiation.

Materials and Methods

Materials

Eagle's minimum essential medium (MEM), horse serum, and antibiotics (penicillin-streptomycin solution and fungizone) were obtained from Gibco Laboratories. Tissue culture dishes and 24-well cluster plates were from Nunc. [^{35}S]methionine, Na^{125}I and [^{3}H]thymidine were from Amersham and nitrocellulose (NC) papers (pore size: 0.22 μm) were from Schleicher and Schuell. Cholera toxin, trypsin (DPCC-treated), thermolysin, heparin-agarose, gelatin-agarose, and other reagents were from Sigma Chemical Co.

Cell culture

Myoblast cultures were prepared according to the method of O'Neill and Stockdale (1972) with minor modifications. Briefly, breast muscles from 12-day-old chick embryos were dissected out, minced, and digested with 0.1% trypsin for 30 min and dispersed by repeated pipetting. The cells were collected by centrifugation and suspended in MEM medium supplemented with 10% horse serum, 10% chick embryo extracts, and 1% antibiotics. The cells were preplated on collagen-coated dishes for 20 min to remove fibroblasts. The cell suspension was then filtered through a four-fold lens paper to remove undissociated cells. Approximately 5 x 10^5 cells per ml were inoculated onto collagen coated dishes. After 24 hr, the medium was replaced with MEM medium containing 10% horse serum, 2% chick embryo extracts, and 1% antibiotics. The plated cells were placed in a humidified incubator in the atmosphere of 95% air and 5% CO_2 at 37°C

Gel electrophoresis

Discontinuous polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate (SDS-PAGE) was carried out as described by Laemmli (1970). Samples were dissolved in SDS-sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 5% 2-mercaptoethanol, and 0.01% bromophenol blue) and boiled for 3 min. SDS-PAGE was carried out at constant current of 40-50 mA for 5-8 hr. Protein was determined by the procedure of Lowry *et al.* (1951) or Bradford (1976) using bovine serum albumin as a standard.

Fusion index

At appropriate times, the cells were washed three times with phosphate buffered saline (PBS) and fixed in a mixture of ethanol, formaldehyde, and acetic acid (20:1:1, V/V) for 5 min. After rinse with distilled water, the cells were stained with hematoxylin for 5 min and washed in a tap water. The degree of fusion was determined by the ratio of the number of nuclei within the myotubes of three or more nuclei divided by the total

number of nuclei as seen under a microscope at x 200. Ten fields were randomly chosen for each dish.

Immunochemical detection of fibronectin

Myoblast homogenates (80 μg protein) were subjected to electrophoresis in 8-14% gradient polyacrylamide slab gels. After electrophoresis, the proteins were transferred onto NC papers at 45 V for 90 min in a trans-blot apparatus assembled as described by Stott *et al.* (1985). The papers were incubated in 5% (W/V) bovine serum albumin at room temperature for 3 hr and then in the antibody solution at 4°C overnight. They were washed with buffer containing 50 mM Tris-HCl, pH 7.4, 200 mM NaCl, and 0.1% Triton X-100. They were then incubated in [^{125}I]protein A solution (2 x 10^5 cpm/ml). After the incubation, the papers were washed four times with the buffer, dried and exposed to X-ray film for autoradiography.

Purification and iodination of fibronectin and 29-kDa amino-terminal fragment of fibronectin

Fibronectin was purified from horse serum by gelatin-agarose and heparin-agarose affinity chromatography as described by Engvall and Ruoslahti (1977). The 29-kDa amino-terminal fragment was generated by thermolysin digestion and purified by the method of Sekiguchi and Hakomori (1983). Fibronectin (1 mg/ml) in 25 mM Tris-HCl, pH 7.6, containing 0.5 mM EDTA, 50 mM NaCl, and 2.5 mM $CaCl_2$ was digested with 5 μg/ml of thermolysin at 22°C for 2 hr. The digestion was terminated by adding EDTA to a final concentration of 5 mM. The digests in 10 mM sodium phosphate buffer, pH 7.6, containing 0.13 M NaCl and 4.5 mM KCl were applied onto a gelatin-agarose column. The unbound fractions were pooled and loaded onto a heparin-agarose column. The bound materials were eluted with 0.5 M NaCl in the same buffer and dialysed against 10 mM sodium phosphate buffer, pH 7.4. After the dialysis, the peptides were subjected to chromatography on a Sephacryl S-200 column. Fractions containing the 29-kDa amino-terminal fragment were pooled and stored at −70°C for further use. The 29-kDa fragment (20 μg) and fibronectin were iodinated with 1 mCi of Na^{125}I by chloramine T method (Greenwood *et al.*, 1963) to a specific activity of 1 x 10^7 cpm/ μg.

Antibody generation

For generating polyclonal antibody to the 29-kDa fragment of fibronectin, rabbits were immunized with the 29-kDa fragment (300 μg). The first three injections were done subcutaneously on the back and intramuscularly on the leg. The second and third injections were subcutaneously on the back. The antigen was mixed with complete Freund's adjuvant for the first time and with incomplete Freund's adjuvant for the second and third injections. The antibody-producing animal was then bled from ear veins for purification of IgG. IgG was then purified from the antisera using a protein A-Sepharose column.

Membrane protein isolation

Myoblasts were cultured for appropriate periods, washed three times with extraction buffer [0.01 M Hepes-NaOH, pH 7.6, 8.55% (w/v) sucrose, 1 mM EDTA, 0.5 mM dithiothreitol, and 2 mM PMSF], scraped off by a rubber policeman, and harvested by centrifugation at 1800 x g for 10 min. The cells were homogenized by 30 strokes with a tight-fitting Dounce pestle in a cold extraction buffer. After removing the low-speed pellet (300 x g, 10 min), a crude membrane preparation was obtained by higher-speed centrifugation at 20,000 x g for 20 min. To obtain plasma membranes, the pellet was suspended in 0.01 M Hepes-NaOH, pH 7.6, 8.55% sucrose, and 1 mM EDTA, layered on a 8.55/21/40/54% discontinuous sucrose gradient, and centrifuged in a SW 60 rotor at 100,000 x g for 2 hr. The materials at 21/40% sucrose interface were taken out and stored at −70°C The membranes were solubilized in buffer A [0.01 M Hepes-NaOH, pH 7.6, 0.14 M NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 1% (v/v) Nonidet P-40] on ice for 1 hr, and then clarified at 23,500 x g for 25 min.

Binding of [^{125}I]29-kDa fragment or [^{125}I] fibronectin to cultured myoblasts

All binding assays were done in MEM/Hepes

supplemented with 0.2% (w/v) bovine serum albumin (binding medium). Myoblast cultures were rinsed three times with prewarmed MEM and incubated with binding medium, containing the [^{125}I]29-kDa fragment or [^{125}I]fibronectin at 37°C After the incubation, cultures were rinsed four times in ice-cold MEM/Hepes, and the cell layers were either sequentially extracted in 1% deoxycholate, followed by 4% SDS or extracted directly in 4% SDS. The deoxycholate extraction was done in 0.02 M Tris-HCl, pH 8.3, containing 2 mM PMSF, 2 mM EDTA and 2 mM N-ethylmaleimide (NEM). The radioactivity of the extracts was measured using a gamma counter.

Assay of DNA synthesis

0.2 μCi of [^{3}H]thymidine was added to multi-well plate at 24 hr after plating and incubated for 33 hr. The cells were rinsed three times with PBS at room temperature and then rinsed twice with 5% TCA. After 10 min, the cells were dissolved in 100% TCA and the radioactivity in them was counted in a liquid scintillation counter.

Results

Effect of cholera toxin and dbcAMP on myoblast fusion

Treatment of cholera toxin to cultured myoblasts resulted in 30-40% percent increase of fusion in a dose-dependent manner accompanying significant decrease in [^{3}H]thymidine incorporation (Figs. 1 and 2). The specificity of this effect was examined by treating the culture with dbcAMP which is known to be permeable to cell membranes. dbcAMP also increased the myoblast fusion and decreased the [^{3}H]thymidine incorporation in a dose dependent manner. But this effect was never as great as that caused by cholera toxin (Figs. 1 and 2). Cholera toxin treatment to myoblasts produced a small but distinct change in the appearance of myoblasts. The cells were more elongated, more obviously lined up end to end and rounding cells were disappeared in cholera toxin-treated culture (data not shown).

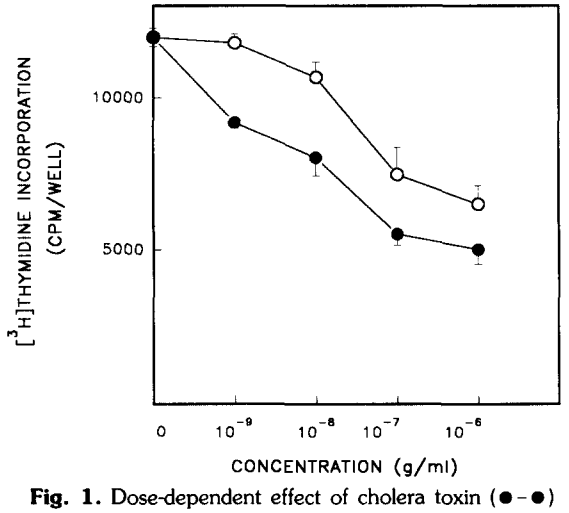

Fig. 1. Dose-dependent effect of cholera toxin (●-●) and dbcAMP (○-○) on the proliferation of myoblasts. Various amounts of cholera toxin and dbcAMP were treated to cultured myoblasts. The rate of [^{3}H]thymidine incorporation was measured as described under Materials and Methods. Each point represents the mean ± S.E.

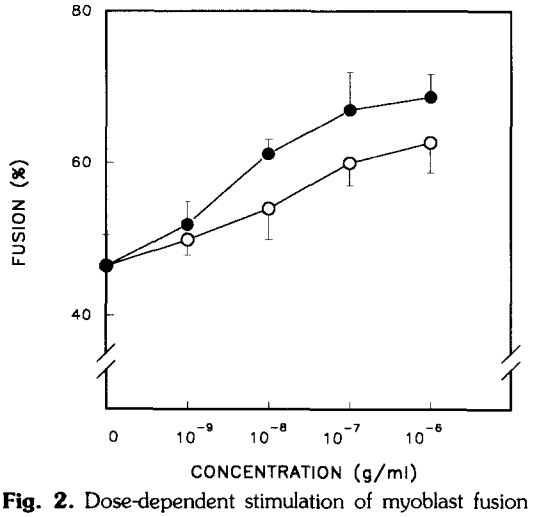

Fig. 2. Dose-dependent stimulation of myoblast fusion by cholera toxin (●-●) and dbc AMP (○-○). Cells were exposed to various amounts of cholera toxin and dbcAMP and scored for percent fusion at 40 hr after the treatment. Each point represents the mean ± S.E.

Effect of cholera toxin and dbcAMP on the binding of 29-kDa fragment and on fibronectin deposition in extracellular matrix

Chick myoblasts were exposed for 2 hr to various concentrations of cholera toxin which causes increased intracellular cAMP levels by specific ADP-ribosylation of G protein. The 29-kDa fragment binding activity was decreased by cholera toxin treatment in a dose-dependent manner. Reduction of 29-kDa fragment binding activity was not complete and the binding reached to an extent of only 20-30% of control (Fig. 3B).

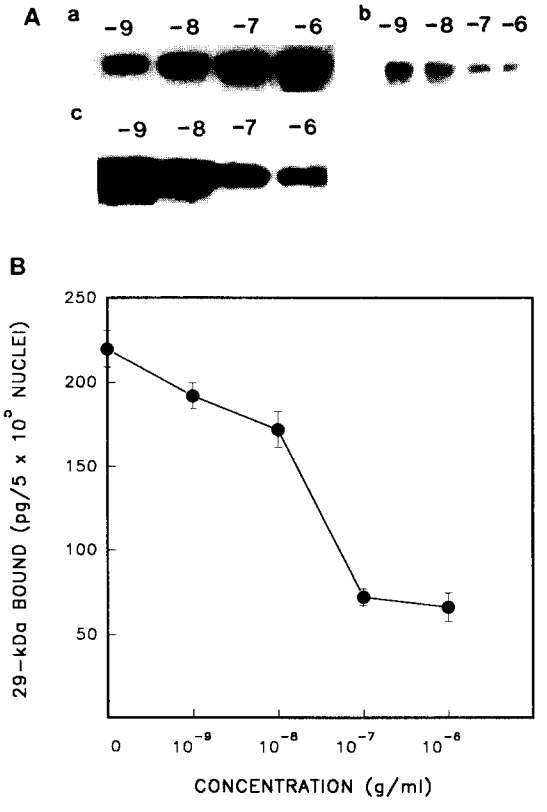

Fig. 3. Alteration of fibronectin in myoblasts at various concentrations of cholera toxin. (A) Immunoautoradiography of fibronectin in pool I (a), pool II (b) and membrane fraction (c). Numerals at the top represent the concentration (g/ml) of cholera toxin. (B) Dose-dependent effect of cholera toxin on the binding of [^{125}I]29-kDa fragment to myoblasts. Binding assay was done at 36 hr after plating as described under Materials and Methods. Each point represents the mean ± S.E.

In order to learn whether the decrease of 29-kDa binding activity decreases the amount of fibronectin in cell surface as well as the incorporation of fibronectin into extracellular matrix, immunoblotting was performed using anti-fibronectin antibody. The cell surface and extracellular matrix fibronectins were discriminated on the basis of their deoxycholate solubility. Deoxycholate soluble fraction contains cell surface and intracelluar fibronectin (pool I) whereas deoxycholate insoluble fraction contains extracellular matrix fibronectin (pool II). The cholera toxin increased fibronectin in pool I but decreased fibronectin in pool II in a dose-dependent manner (Fig. 3A). These results were not consistent with that obtained from [^{125}I]29-kDa fragment binding assay (Fig. 3B).

To clarify these contradiction, pool I is further fractionated into membrane fraction and cytoplasmic fraction and then the membrane fraction was also immunoblotted with anti-fibronectin antibody. Fibronectin of membrane fraction decreased in cholera toxin-treated cells (Fig. 3A-c). This result implies that the increase of fibronectin in pool I is not due to the increase of matrix assembly site which interacts with the 29-kDa fragment of fibronectin but to endogenous fibronectin synthesis. Similarly, the decrease of the amount of fibronectin in pool II appears to be resulted from the decrease of matrix assembly site in the membrane fraction. The results that cholera toxin increases the endogenous fibronectin synthesis are consistent with the studies of Dean *et al.*, (1990) that cAMP increases the endogenous fibronectin synthesis.

The cellular receptor for cholera toxin is the oligosaccharide moiety of the ganglioside GM1 (Yamada *et al.*, 1983). Gangliosides have been reported to mediate the attatchment of some, but not all, cells to fibronectin and to facilitate the organization of fibronectin into a fibrillar network (Matyas *et al.*, 1986). Therefore, it is likely that the cholera toxin-induced decrease in [^{125}I] fibronectin binding is a consequence of the decrease in the number of cell surface gangliosides rather than the decrease of matrix assembly receptor. Thus, dbcAMP was tested for its effect on binding activity. dbcAMP also decreased the

amount of fibronectin in extracellular matrix (Fig. 4). Therefore, we suggest that intracellular cAMP does influence on the fibronectin binding activity of myoblast cell surface and the decrease in binding activity observed with cholera toxin is not due to a side-effect.

Effect of RGDS peptide on the proliferation of myoblasts.

The above results suggested that cAMP may act to inhibit the proliferation of chick myoblast by decreasing the 29-kDa binding activity and fibronectin accumulation in extracellular matrix. However, we cannot rule out the possibility that cAMP regulates the myogenesis not by controlling the amount of matrix assembly site but by another signal pathway. Thus, some reagents known to inhibit the matrix assembly of fibronectin were used to prove that the induction of precocious fusion accompanying the reduction of myoblast proliferation was caused by the decrease of matrix

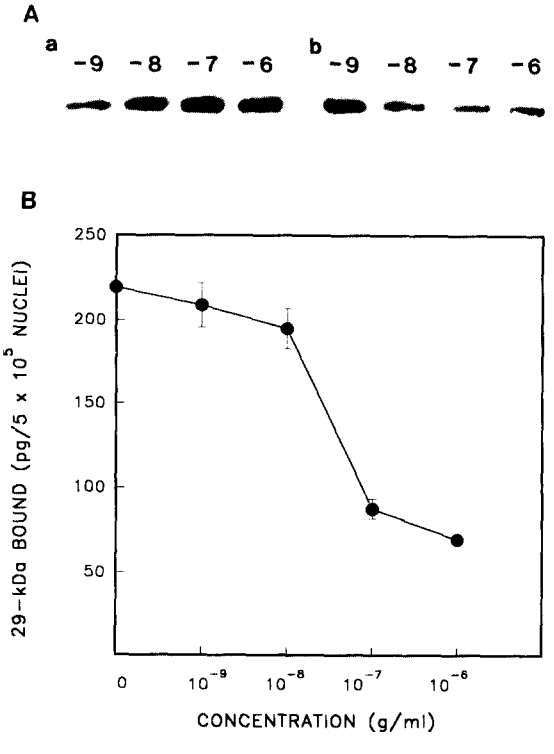

Fig. 4. Alteration of fibronectin in myoblasts at various concentrations of dbcAMP. (A) Immunoautoradiography of fibronectin in pool I (a) and pool II (b). Numerals at the top represent the concentration (g/ml) of dbcAMP. (B) Dose-dependent effect of dbcAMP on the binding of [^{125}I]29-kDa fragment to myoblasts. Binding assay was done at 36 hr after plating as described under Materials and Methods. Each point represents the mean ± S.E.

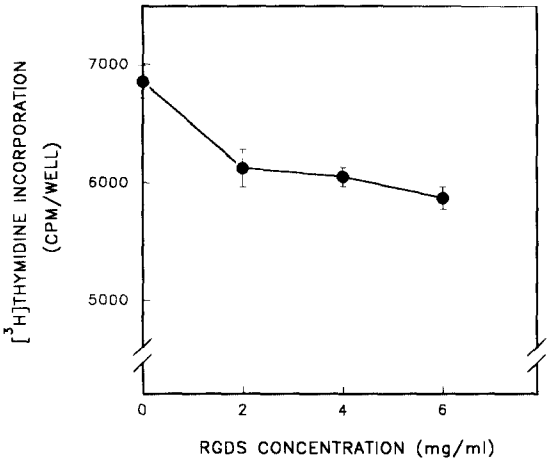

Fig. 5. Dose-dependent effect of the RGDS peptide on the proliferation of myoblasts. The RGDS peptide was added to culture medium at 24 hr after plating. The rate of [^{3}H]thymidine incorporation was measured as described under Materials and Methods. Each point represents the mean ± S.E.

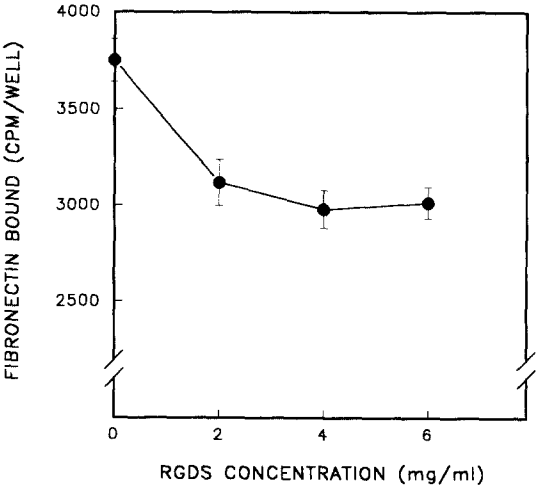

Fig. 6. Dose-dependent effect of the RGDS peptide on the incorporation of [^{125}I]fibronectin into extracellular matrix. Binding assay was done at 36 hr after plating as described under Materials and Methods. Each point represents the mean ± S.E.

assembly sites for fibronectin. The RGDS peptide that inhibits the matrix assembly of fibronectin was treated to the myoblasts. The RGDS peptide also inhibited the cell proliferation in a dose-dependent manner, maximum inhibition of cell proliferation being only 30-40% of cholera toxin (Fig. 5). Since this difference might be caused by a difference of binding affinity between the RGDS and fibronectin to myoblasts, we assumed that the RGDS do not completely inhibit the incorporation of fibronectin into extracellular matrix. This assumption was confirmed by Fig. 6, which showed that the RGDS partially inhibits the binding of [^{125}I]fibronectin into extracellular matrix.

Discussion

Previous studies have demonstrated that extracellular matrix regulates the proliferation, growth, and differentiation of myoblasts. Von der Mark and Ocalan (1986) suggested that fibronectin may stimulate the proliferation of replicating presumptive myoblasts and prevent the cells from differentiation. In contrast, laminin stimulates the proliferation of myogenic cell and the majority of cells differentiate into postmitotic, fusion-capable myoblasts. During the muscle morphogenesis, the formation of myotubes in certain spatial arrangement may be regulated by fibronectin-containing matrix produced by connective tissue cells (Chiquet *et al.*, 1981). Immunocytochemical observation on the L6 muscle cell line and cloned human muscle cells indicates that as myoblasts fuse they lose their surface fibronectin (Walsh *et al.*, 1981). This decrease of fibronectin during myogenesis is due to the matrix assembly site which interacts with 29-kDa fragment of fibronectin on myoblast cell surface (Chung and Kang, 1990).

In fibroblasts, cholera toxin decreases the matrix assembly site and incorporation of fibronectin into extracellular matrix (Allen-Hoffmann and Mosher, 1987). On the other hand, increase in cAMP levels has been found to coincide with fusion in primary embryonic chick cultures (Zalin and Montague, 1974). Cholera toxin also induces the precocious fusion of rat myoblasts (Stygall and Mirsky, 1980). To our knowledge, however, no experiment has been performed to explain the relationship between the signal molecule regulating the matrix assembly of fibronectin and myogenesis. Thus, it is important to identify cellular control mechanism that might account for the alteration of matrix assembly site during myogenesis and also to reveal the role of fibronectin during myogenesis.

In the present study, we found that cholera toxin treated to myoblasts inhibits the proliferation but induces the precocious fusion of chick myoblasts, accompanied by the decrease of fibronectin on cell surface. Since cholera toxin specifically raises cAMP levels and its effect can be mimicked to a large extend by adding dbcAMP, it is likely that cAMP regulates the myogenesis through controlling fibronectin accumulation in extracelluar matrix. We also found that the RGDS peptide inhibits the proliferation of chick myoblasts. It has been known that the process of fibronectin matrix assembly occurs in two steps. First, fibronectin binds to $\alpha5\beta1$ integrin through the RGDS sequence which is located in cell binding domain of fibronectin. Second, the fibronectin bound to $\alpha5\beta1$ is then incorporated into the extracellular matrix by interaction of N-terminal fragment of fibronectin with matrix assembly receptor (Chuanyue *et al.*, 1993).

The RGDS peptide is known to bind competitively to $\alpha5\beta1$ with fibronectin in the first step, inhibiting the matrix assembly of fibronectin. Furthermore, we previously suggested that amino-terminal fragment of fibronectin inhibits the proliferation of myoblasts (Moon *et al.*, 1992). Therefore, the finding that the RGDS peptide inhibits the proliferation of myoblasts may confirm that the amount of fibronectin in extracellular matrix regulates the cell cycle of myoblats. However, we are unable to address the question how fibronectin regulates the cell cycle and differentiation by the present findings. Ingber *et al.* (1990) suggested a new model for the mechanism by which fibronectin regulates the cell proliferation. They proposed that fibronectin alters cytoplasmic pH as a result of specific binding interactions with a member of the integrin family and that changes of cytoplasmic pH modulate the

proliferation of capillary endothelial cells. The changes of pH and growth-modulating effects of fibronectin may be based, at least in part, on its ability to support the changes of cell shape. In the case of chick myoblasts, membrane fusion is accompanied by the change of cell shape which exerts mechanical tension on various cytoskeletons and membrane components. It is likely that the cell shape change, as a result of the decrease of fibronectin in extracellular matrix, triggers a cellular signal pathway. This possibility is supported by the observation that stretch activated Ca^{2+} channels exist on myoblasts (Fransco and Lansman, 1990) and that integrin-fibronectin interaction hyperpolarizes the membrane potential by activating the K^+ channels (Archangeli, 1993).

Based upon our observations and others, we propose that fibronectin, which is regulated by cAMP, appears to control the proliferation of myoblasts by modulating the cell shape. This is just what would be expected if fibronectin acts not on the process of fusion per se, but reduces the frequency by which postmitotic, fusion-capable cells emerge from the proliferative population.

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References

Akiyama, S.K., S.S. Yamada, W.T. Chen, and K.M. Yamada, 1989. Analysis of fibronectin receptor function with monoclonal antibodies: Roles in cell adhesion, migration, matrix assembly receptor, and cytoskeletal organization. *J. Cell Biol.* **109:** 863-875.

Allen-Hoffmann, B.L., and D.F. Mosher, 1987. Matrix assembly sites for exogenous fibronectin are decreased on human fibroblsats after treatment with agents which increase intracellular cAMP. *J. Biol. Chem.* **262:** 14361-14365.

Arcangeli, A., A. Becchetti, A. Mannini, G. Mugnai, P.D. Filippi, G. Tarone, E. Barletta, E. Wanke, and M. Olivotto, 1993. Integrin-mediated neurite outgrowth in neuroblastoma cells depends on the activation of potassium channels. *J. Cell Biol.* **122:** 1131-1143.

Bowlus, C.L., J.J. Mcquilan, and D.C. Dean, 1991. Characterization of three different elements in the 5'-flanking region of the fibronectin gene which mediate a transcriptional response to cAMP. *J. Biol. Chem.* **266:** 1122-1127.

Bradford, M.M. 1976. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72:** 248-254.

Chiquet, M., H.M. Eppenberger, and D.C. Turner, 1981. Muscle morphogenesis: Evidence for an organizing function of exogenous fibronectin. *Dev. Biol.* **88:** 220-235.

Chuanyue, W., J.S. Bauer, R.L. Julliano, and J.A. McDonald, 1993. The $\alpha 5\beta 1$ integrin fibronectin receptor, but not the $\alpha 5$ cytoplasmic domain, functions in an early and essential step in fibronectin matrix assembly. J. *Biol. Chem.* **268:** 21883-21888.

Chung, C.Y., and M.S. Kang, 1987. Decrease of surface fibronectin availibility required for myoblast adhesion by tunicamycin. *Korean J. Zool.* **30:** 325-340.

Chung, C.Y., and M.S. Kang, 1990. Correlation between fibronectin and its receptor in chick myoblast differentiation. *J. Cell. Physiol.* **142:** 392-400.

Dean, D.C., J.J. McQuilan, and S. Weintraub, 1990. Serum stimulation of fibronectin gene expression appears to result from rapid serum-induced binding of nuclear proteins to a cAMP response element. *J. Biol. Chem.* **84:** 1876-1880.

Engvall, E., and E. Ruoslahti, 1977. Binding of soluble form of fibroblast surface protein, fibronectin, to collagen, *Int. J. Cancer* **20:** 1-5.

Fransco, A., and J.B. Lansman, 1990. Strectch-sensitive channels in developing muscle cells from a mouse cell line. *J. Physiol.* **427:** 361-380.

Gardner, J.M., and D.M. Fambrough, 1983. Fibronectin expression during myogenesis. *J. Cell Biol.* **96:** 474-485.

Greenwood, F.C., W.M. Hunter, and J.S. Glover, 1963. The preparation of ^{131}I-labeled human growth hormone of high specific radioactivity. *Biochem. J.* **89:** 114-132.

Ingber, D.E., D. Prusty, J.V. Frangioni, E.J. Cragoe, and M.A. Schwartz, 1990. Control of intracellular pH and growth by fibronectin in capillary endothelial cells. *J. Cell Biol.* **110:** 1803-1811.

Knuden, K.A., and A.F. Horwitz, 1977. Tandem events in myoblast fusion. *Dev. Biol.* **58:** 328-338.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4.

Nature **227:** 680-685.

Lowry, O.H., N.G. Rosenberg, A.L. Farr, and R.J. Randall, 1951. Protein measurement with the folin reagent. *J. Biol. Chem.* **193:** 265-275.

Matyas, G.R., D.C. Evers, R. Radinsky, and D.J. Morre, 1986. Fibronectin binding to gangliosides and rat liver plasma membranes. *Exp. Cell Res.* **162:** 296-318.

McDonald, J.A., B.J. Quade, T.J. Broeklmann, R. LaChance, K. Forsman, E. Hasegawa, and S. Akiyama, 1987. Fibronectin's cell adhesive domain and an amino-terminal matrix assembly domain participate in its assembly into fibroblast pericellular matrix. *J. Biol. Chem.* **262:** 2957-2967.

McKeown-Longo, P.J., and D.F. Mosher, 1985. Interaction of the 70,000-mol-wt amino terminal fragment of fibronectin with the matrix assembly receptor of fibroblasts. *J. Cell Biol.* **100:** 364-374.

Moon, K.Y., K.S. Shin, and M.S. Kang, 1992. Alteration of matrix assembly receptor for fibronectin during chick myogenesis. *Mol. Cells.* **2:** 265-271.

Olwin, B.B., and Z.W. Hall, 1985. Developmental regulation of laminin accumulation in the extracelluar matrix of a mouse muscle cell line. *Dev. Biol.* **112:** 359-367.

O'Neill, M.C., and F.B. Stockdale, 1972. A kinetic analysis of myogenesis in vitro. *J. Cell Biol.* **52:** 52-65.

Pierschbacher, M.D., and E. Ruoslahti, 1984. The cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. *Nature* **309:** 30-33.

Podleski, T.R., I. Greenberg, J. Schlessinger, and K.M. Yamada, 1979. Fibronectin delays the fusion of L6 myoblasts. *Exp. Cell Res.* **122:** 317-326.

Sekiguchi, K., and S. Hakomori, 1983. Domain structure of human plasma fibronectin. *J. Biol. Chem.* **258:** 3967-3973.

Stott, D.J., J. McLearie, and H.S. Marsden, 1985. A gel transfer tank for immunoblotting and its application for analysis of nuclear protein antigen. *Anal. Biochem.* **149:** 454-460.

Stygall, K., and R. Mirsky, 1980. The effect of cholera toxin on myogenesis in rat skeletal muscle cultures. *Dev. Biol.* **78:** 14-24.

Von der Mark, K., and M. Ocalan, 1986. Antagonistic effects of laminin and fibronectin on the expression of the myogenic phenotype. *Differentiation* **40:** 150-157.

Walsh, F.S., S.E. Moore, and S. Dhut, 1981. Specific changes in cellular glycoproteins and surface proteins during myogenesis in clonal muscle cells. *Dev. Biol.* **84:** 121-132.

Yamada, K.M., D.R. Critchley, P.H. Fishman, and J. Moss, 1983. Exogenous gangliosides enhance the interaction of fibronectin with ganglioside-deficient cells. *Exp. Cell Res.* **143:** 295-302.

Zalin. R.J., and W. Montague, 1974. Changes on adenylate cyclase, cyclic AMP and protein kinase levels in chick myoblasts, and their relationship to differentiation. *Cell* **2:** 103-107.

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계배 근원세포의 분화에서 extracellular matrix내 fibronectin의 역할

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근원세포의 분화과정에서 fibronectin의 양의 감소는 fibronectin amino-terminal의 29-kDa fragment에 대한 matrix assembly receptor의 감소에 기인한다고 보고된 바 있다. 본 연구에서는 이러한 fibronectin의 양적 감소가 G protein과 adenylate cyclase 활성도에 의해 조절을 받으며 fibronectin level의 감소는 근원세포의 증식을 감소시켜 precocious fusion을 유도한다는 증거를 얻었다. 그 증거로는 1) 세포내 cAMP의 농도를 증가시키는 cholera toxin은 근원세포에서 fibronectin과 matrix assembly receptor의 양을 감소시켰다. 2) Cholera toxin은 근원세포의 증식을 감소시켜 precocious fusion을 유도하였다. 3) 근원세포의 증식을 억제하고 분화를 촉진시킨 dbcAMP는 fibronectin과 matrix assembly receptor의 양도 감소시켰다. 4) Fibronectin이 extracellular matrix로 축적되는 것을 막는다고 알려진 RGDS sequence는 근원세포의 증식을 억제하고 분화를 촉진시켰다. 이러한 결과들은 근원세포에서 cAMP는 fibronectin과 matrix assembly receptor의 양적 변화를 조절하고 fibronectin의 양적 변화는 근원세포의 증식과 분화를 조절함을 뒷받침하는 것이다.