Characterization of Insulin-Like Growth Factor I Receptors on Madin-Darby Canine Kidney (MDCK) Cell Line

IZUMI SUKEGAWA, NAOMI HIZUKA, KAZUE TAKANO,
KUMIKO ASAKAWA AND KAZUO SHIZUME

*Department of Medicine
Institute of Clinical Endocrinology
Tokyo Women's Medical College, Tokyo 162, Japan*

Abstract

Specific insulin-like growth factor I (IGF-I) receptors on the Madin-Darby canine kidney (MDCK) cell line were identified and characterized. [125 I]IGF-I specifically bound to the cells, but [125 I]insulin bindings to the cells was minimal. Unlabeled IGF-I displaced both the IGF-I and insulin bindings with potencies that were 100 and 10 times as great as insulin. By an affinity labeling technique, IGF type I receptors were present in the MDCK cells. IGF-I stimulated DNA synthesis and cell proliferation at physiological concentrations. On the other hand, insulin had a little effect on DNA synthesis. These data suggest that IGF type I receptors as demonstrated in MDCK cells are involved in DNA synthesis and cell proliferation.

Specific receptors for insulin-like growth factor (IGF) and insulin are located on the cell membrane of a variety of cells (Nissley and Rechler, 1984) and both receptors co-exist in most cells. Both hormones have similar biological activities and it is known that each hormone can bind to the receptor of the other.

The MDCK cell line, established from dog kidney (Madin and Darby, 1958), has the morphological properties of distal tub-

ular epithelial cells (Rindler *et al.*, 1979; Boerner and Saier, 1985) and it also possesses some functional properties similar to those of normal kidney (Lin *et al.*, 1982 and 1985; Bechner *et al.*, 1985). While insulin-like effects were produced in this cell line by insulin mimickers, such as concanavalin A and wheat germ agglutinin (Roth *et al.*, 1981), Hofmann *et al.*, (1985) reported that insulin receptors were not present in the MDCK cell line. Recently, Krett *et al.*, (1986) reported that IGF type I and II receptors were present in the cell line.

In the present study, we have characterized IGF-I receptors on the MDCK cell line and investigated its mitogenic effects on the cells.

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Please address all correspondence to:

Dr. Izumi Sukegawa
Department of Medicine,
Institute of Clinical Endocrinology
Tokyo Women's Medical College
8-1 Kawada-cho, Shinjuku-ku
Tokyo, 162, Japan

Materials and Methods

Cells

Cultured MDCK cell line (originally from the American Type Culture Collection) was provided by Dr. Nakajima, of the Institute of Medical Science, University of Tokyo. The cells were grown at 37°C in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS) under 5% CO₂-95% air atmosphere.

Hormones and reagents

IGF-I was kindly provided by Fujisawa Pharmaceutical Co. (Osaka, Japan). The IGF-I preparation was synthesized by recombinant DNA technology as described (Niwa *et al.*, 1986), and the amino acid sequence of the preparation is the same as that of natural IGF-I. Crystalline porcine insulin was a gift from Eli Lilly Co. (Indianapolis, IN). Multiplication stimulating activity (MSA) was purchased from Collaborative Research (Lexington, MA), and ¹²⁵I Na and [³H] thymidine from New England Nuclear (Boston, MA). IGF-I and insulin were iodinated by a chloramine-T method modified as reported (Zapf *et al.*, 1975; Roth, 1975) with a specific activity of 180 µCi/µg and 100 µCi/µg, respectively.

Binding studies for IGF-I and insulin

The subconfluent MDCK cells were washed with phosphate-buffered saline (PBS) and incubated for detachment with 0.02% EDTA and 0.05% trypsin for 10 min at 37°C. The cells were suspended in assay buffer (Buffer G) (Gambhir *et al.*, 1978) and incubated with [¹²⁵I]IGF-I (0.15–0.2 ng/ml) in the presence or absence of IGF-I in a total volume of 0.5 ml. At the end of the incubation, replicate 200 µl aliquots were layered onto ice-cold buffer and centrifuged for 2 min at 8000×g in a Beckman B microfuge (Beckman, Palo Alto, CA). After aspirating the supernatant, cell-associated radioactivity was counted in a γ-spectrometer. Nonspecific binding was defined as cell-associated radioactivity in the presence of an excess of unlabeled IGF-I (1 µg/ml). Specific binding is the difference between total binding and nonspecific binding. The binding study for insulin was carried out similarly to that for IGF-I.

[³H]Thymidine incorporation into DNA

The cells (0.4×10⁵ cells/ml) were plated to

multi-well dishes supplemented with DMEM containing 10% FCS and incubated for 24 h, and then the cells (1×10⁵ cells/ml) were cultured in serum-free DMEM containing 0.25% bovine serum albumin (BSA) for 24 h at 37°C. The cells were incubated with or without IGF-I and/or insulin for 18 h at 37°C, followed by the pulse with [³H]thymidine (0.5 µCi) for 1 h at 37°C. The cells were washed twice with ice-cold PBS with 0.25% BSA, and the trichloroacetic acid (TCA) precipitable [³H]thymidine was counted in a scintillation spectrometer.

Cell proliferation

MDCK cells were plated as described above, and then the cells were washed twice with DMEM containing 1% FCS and incubated with IGF-I (0–100 ng/ml). The number of cells was counted every day with a Coulter Counter (Coulter Electronics, Hialeath, FL).

Cross-linking of [¹²⁵I]IGF-I with MDCK cells

The MDCK cells (4×10⁷ cells/ml) were incubated in 50 mM N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid (HEPES) lymphocyte buffer (Eastman *et al.* 1979) with 0.1% BSA at 4°C overnight with [¹²⁵I]IGF-I in the presence or absence of unlabeled IGF-I (1 µg/ml). The cells were washed in ice-cold buffer without BSA and resuspended in the same buffer. Disuccinimidyl suberate (DSS), dissolved in dimethyl sulfoxide, was added at a final concentration of 1 mM. After 30 min, the reaction was stopped by 10 mM Tris-(hydroxymethyl) aminomethane and 1 mM EDTA. The cells were solubilized in 50 mM HEPES buffer containing 1% Triton X-100, 0.15 M NaCl and 2 mM phenylmethylsulfonyl fluoride for one hour at 4°C and centrifuged at 100,000×g for 45 min. The supernatant was precipitated with ice-cold 10% TCA and the pellet was boiled for 5 min in 2% sodium dodecyl sulfate (SDS), 10% (v/v) glycerol, 0.5 M 2-mercaptoethanol, 0.01% bromophenol blue and 10 mM sodium phosphate buffer, pH 7.0. Samples were analyzed by SDS/polyacrylamide gel electrophoresis (PAGE) according to Laemmli (1970). Autoradiography was carried out using Kodak X-Omat film with an intensifying screen.

Results

Binding of [125 I]IGF-I to MDCK cells

[125 I]IGF-I specifically bound to the MDCK cells, and the binding increased linearly as a function of the concentration. The binding of [125 I]IGF-I to the cells was

also time- and temperature-dependent (Fig. 1). At 4°C, the binding reached a plateau by 24 h, and the maximal binding was greater than that at 37°C or 15°C. Binding studies were therefore performed at 4°C for 24 h. At a cell concentration of 6×10^6 cells/ml, the specific binding of [125 I]IGF-I averaged 12% of the total [125 I]IGF-I ad-

Fig. 1. Effect of time and temperature on specific [125 I]IGF-I binding to the MDCK cells. The cells (6×10^6 cells/ml) were incubated at 37°C, 15°C, and 4°C for various lengths of time.

Fig. 2A. Competition for specific [125 I]IGF-I binding to the MDCK cells (3.6×10^6 cells/ml) by IGF-I, multiplication stimulating activity (MSA) and porcine insulin.

ded. The nonspecific binding was less than 15% of total binding.

Specificity of IGF-I binding

[¹²⁵I]IGF-I binding to the MDCK cells was displaced by unlabeled IGF-I in a dose-dependent manner (Fig. 2A). A 50% inhibition of [¹²⁵I]IGF-I binding was obtained at a concentration of 10 ng/ml IGF-I. The Scatchard analysis for this binding revealed a curvilinear pattern (Fig. 2B). Assuming two classes of binding sites, the affinity constant (K_a) and the number of binding sites per cell for high affinity binding site were $9.5 \times 10^8 \text{ M}^{-1}$ and 34,000, respectively. The binding of [¹²⁵I]IGF-I to the cells was also displaced by MSA and porcine insulin, with potencies 10 and 100 times less than that of IGF-I, respectively (Fig. 2A). In addition, [¹²⁵I]insulin binding to the cells was minimal, and IGF-I displaced the [¹²⁵I]insulin binding with 10 times greater potency than insulin (Fig. 3). When the MDCK cells were cross-linked to [¹²⁵I]IGF-I with DSS, solubilized and electrophoresed in the presence of reductant, the major band migrated at $M_r=130,000$. An excess of unlabeled IGF-I blocked the formation of this band (Fig. 4).

Effect of IGF-I on DNA synthesis

IGF-I stimulated [³H]thymidine incorporation into DNA at a concentration as low as 0.1 ng/ml. Maximal stimulation was observed at 10 ng/ml IGF-I with the thymidine

Fig. 2B. Scatchard analysis of [¹²⁵I]IGF-I binding to the MDCK cells. B/F, Bound to free ratio.

Fig. 3. Competition for total [¹²⁵I]insulin binding to the MDCK cells (1×10^7 cells/ml) by porcine insulin and IGF-I.

Fig. 4. Cross-linking of [¹²⁵I] IGF-I with MDCK cells.

Left lane: unlabeled IGF-I (—), Right lane: unlabeled IGF-I (+).

incorporation being 260% of the control (Fig. 5). A half-maximum stimulation was achieved at a concentration of 0.7 ng/ml. On the other hand, insulin (10 μ g/ml) had

little, if any, effect on DNA synthesis with 118% of control, while both IGF-I and insulin in maximally effective concentrations had no additive effect (data not shown).

Fig. 5. Stimulation of [³H]thymidine incorporation into DNA synthesis in MDCK cells (1×10^5 cells/ml) by IGF-I.

Fig. 6. The number of MDCK cells grown for 2 days in DMEM with 1% FCS in the presence of various concentrations of IGF-I (0–100 ng/ml). Values represent the mean $\pm$ SEM for four experiments.

Effect of IGF-I on cell proliferation

When IGF-I was present in the cell culture with 1% FCS for two days, the number of cells increased as a function of the concentration of IGF-I (Fig. 6). At 100 ng/ml, the number of cells increased to $162 \pm 11.6\%$ (mean $\pm$ SEM, $n=4$) of control. Even at a physiological concentration (1.0 ng/ml), IGF-I significantly stimulated cell proliferation.

Discussion

In the present study, specific IGF-I receptors on the MDCK cell line were identified and characterized. [¹²⁵I]IGF-I specifically bound to the MDCK cells and the binding characteristics were similar to those demonstrated in other cells. Compared to

IGF-I, the relative potencies of MSA and insulin in displacing [¹²⁵I]IGF-I binding were 10 and 1%, respectively. The IGF-I binding subunit was $M_r=130,000$ in an affinity-labeling technique. These results indicate that IGF type I receptors are present in these cells, confirming the previous data of Krett *et al.* (1986).

In contrast, [¹²⁵I]insulin binding to the cells was minimal, and IGF-I inhibited [¹²⁵I]-insulin binding 10 times more than insulin. This result suggests that [¹²⁵I]insulin binds to IGF-I receptor but not to insulin receptor.

At a physiological concentration, IGF-I markedly stimulated DNA synthesis in the MDCK cells and increased the number of cells, indicating that IGF-I binding sites are functionally important in mediating its mitogenic effect. However, insulin even at a pharmacological concentration scarcely

stimulated DNA synthesis. These data suggest that IGF-I and insulin act on the MDCK cells through IGF type I receptors.

In summary, this study confirms that IGF-I receptors, but not insulin receptors, are present on the MDCK cells, and further demonstrates that the IGF-I has biological effects on the cells. These data imply that the presence of specific IGF-I receptors may be functionally important for the cells and that this cell line may be useful for future studies concerning the interaction between IGF-I and its receptor.

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