Construction of an *in vitro* allergy reaction evaluation system using human leukemia cell lines

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Abstract

Human leukemia cell lines, KU812 and KU812F, are immature prebasophilic cell lines and have a potential to differentiate into basophils. Hydrocortisone (HC) and sodium nitroprusside (SNP) can enhance the cell surface FcεRI expression of KU812 cells. However, the differentiated phenotypes of KU812 cells were unstable, hindering the application of KU812 cells to construct a practical *in vitro* allergy reaction evaluation system. Here, we attempted to enhance the cell surface expression of FcεRI on hydrocortisone (HC)- or sodium nitroprusside (SNP)-treated KU812 cells by IgE. The cell surface FcεRI expression was observed in about 20, 20 and 26% of 1 μM HC-, 1 nM SNP- and 450 ng ml⁻¹ IgE-treated KU812 cells, respectively. Whereas, the cell surface FcεRI expression was observed in about 54% of KU812 cells treated with both 450 ng ml⁻¹ IgE and 1 μM HC for 8 days, and in about 33% of KU812 cells treated with both 450 ng ml⁻¹ IgE and 1 nM SNP for 4 days. Ninety five% of the IgE/HC- or IgE/SNP-treated KU812 cells expressed CD 13 antigen, a cell surface marker of basophils. An electrophoretic mobility shift assay revealed that AP-1, NF-AT and NF-κB transcription factors were all activated in IgE/HC- and IgE/SNP-treated KU812 cells. Since the differentiated KU812F cells were more sensitive than KU812 cells for histamine release by sensitization with human IgE and anti-IgE antibody, a practical *in vitro* allergy reaction evaluation system for general use was constructed using IgE/HC-treated KU812F cells. The differentiated KU812F cells sensitized with an allergic patient's IgE and mite allergen exhibited histamine release. The constructed *in vitro* allergy reaction evaluation system using differentiated human leukemia KU812F cells will be useful to study allergic reaction and to analyze physiologically functional substances in allergic disease.

Abbreviations: BSA, bovine serum albumin; DTT, dithiothreitol; EDN, eosinophil-derived neurotoxin; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; EMSA, electrophoretic mobility shift assay; EPO, eosinophil peroxidase; HC, hydrocortisone; HEPES, hydroxyethylpiperazinylethanesulfonic acid; HPLC, high performance liquid chromatography; β-LG, β-lactoglobulin; mAb, monoclonal antibody; OPA, o-phthalaldehyde; OVA, ovalbumin; PBS, phosphate buffered saline; PMSF, phenylmethanesulfonyl fluoride; SNP, sodium nitroprusside.

Introduction

Human basophils and mast cells, which are activated by immunoglobulin E (IgE) and allergen, play the important role in allergic disorders such as asthma, atopic dermatitis and allergic rhinitis. A cross-linking of two adjacent FcεRI molecules attached allergen-

specific IgE by a binding of polyvalent allergen triggers degranulation, resulting in a release of mediators including histamine, leukotrienes and prostaglandins. This sequence of events leads to the allergic inflammation (Drombrowicz et al., 1993; Gauchat et al., 1993; Metzger, 19991; Yanagihara et al., 1997). Thus, the activation of basophils and mast cells through FcεRI

is indispensable to initiate allergic reactions. However, human origin basophils or mast cell lines, which possess physiological functions, are not available now for *in vitro* assay. Most of studies about signaling cascades and functionalities of mast cells and basophils are obtained from non-human origins. Human mast cells and basophils can be induced by culturing multipotent hematopoietic stem cells in the presence of IL-13, stem cell factor and IL-6 (Boyce et al., 1995; Saito et al., 1996; Toru et al., 1996; Nilsson et al., 1994). To analyze signaling cascades and functionalities *in vitro* of the allergic inflammation reaction mediated by mast cells and basophils, it is desirable to use human-derived cell lines. A human prebasophilic cell line, KU812, derived from human chronic myelocytic leukemia, has the potential to differentiate into mature hematopoietic cells including basophils. The KU812 cell line was thought to be a desirable cell line for analysis of human allergic inflammation reaction in connection with the expression of Fc ϵ RI chain and CD40 mRNAs and the production of histamine (Blom et al., 1992; Kishi et al., 1985; Hara et al., 1998; Magnusson et al., 1995; Navarro et al., 1990; Taudou et al., 1993; Nilsson et al., 1994; Valent et al., 1990). However, KU812 cells express Fc ϵ RI on the cell surface at a low level as compared to basophils *in vivo* and the differentiated phenotypes of KU812 cells were unstable, hindering the application of the cell line to construct a practical *in vitro* allergy reaction evaluation system for general use.

Thus, sufficient induction of the differentiation was needed to utilize the KU812 cells as a model of human basophils. Hara et al. (1998, 2000) reported that hydrocortisone (HC), an endogenous glucocorticoid, could enhance the cell surface expression of Fc ϵ RI, and increased histamine content by the treatment of interleukin (IL)-4 and the differentiated KU812 cells released histamine on IgE-cross-linking. Moreover, it has been reported that IgE could enhance the Fc ϵ RI expression and the release of histamine, exhibiting a synergistic effect with IL-4 using human or mouse mast cells (Xia et al., 1997; Yamaguchi et al., 1997, 1999). Recently, Yamashita et al. (2001) reported that the treatment of KU812 cells with HC and sodium nitroprusside (SNP) differentiated themselves into basophilic and eosinophilic cells, respectively. Here, we attempted to enhance the Fc ϵ RI expression in KU812 cells by combined use of HC or SNP with IgE and constructed a practical *in vitro* allergy reaction evaluation system using IgE/HC-treated KU812F cells. The differentiated KU812F cells released histamine

on sensitization with allergic patient's IgE and mite allergen.

Materials and methods

Reagents

HC, SNP, sodium citrate, boric acid, 2-mercaptoethanol, Brij-35, dithiothreitol (DTT) and o-phthalaldehyde (OPA) were purchased from Wako (Kyoto, Japan). Phenylmethanesulfonyl fluoride (PMSF), leupeptin and poly (dI-dC) poly (dI-dC) were purchased from Sigma (St. Louis, MO, U.S.A.). The protein assay kit was purchased from Bio-Rad Lab. (Richmond, CA, U.S.A.). End-labelled [γ -³²P] dATP was purchased from Amersham Pharmacia Biotech. (Buckinghamshire, U.K.). Anti-human Fc ϵ RI antibody (CRA-I) was purchased from Kyokuto (Tokyo, Japan). Mouse IgG antibody, FITC-conjugated anti-mouse Ig's, anti-human IgE and FITC-conjugated goat anti-mouse IgM antibody were purchased from TAGO (Burlingame, CA, U.S.A.). Human myeloma IgE and mite allergen were purchased from Cosmo-Bio Co. (Tokyo, Japan). Mouse anti-human CD13 monoclonal antibody (mAb) (IgG type) was purchased from YLEM S. R. L. (Roma, Italy). Mouse anti-human CD15 mAb (IgM type) was purchased from Pharmingen (San Diego, CA). PE-conjugated goat anti-mouse IgG antibody was purchased from IMMUNOTECH (Marseille, France).

Cells and cell culture

Immature prebasophilic cell lines, KU812 and KU812F, were obtained from Japan Collection of Research Bioresources (JCRB) for induction of the differentiation and the release of histamine, and we used a human lymphocyte cell line, U266B1 cells (ATCC #TIB-194), to produce IgE antibody. The cells were maintained in a RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine albumin, 2 mM L-glutamine, 10 mM hydroxyethyl-piperazinylethanesulfonic acid (HEPES) buffer and antibiotics (100 U ml⁻¹ penicillin G and 100 μ g ml⁻¹ streptomycin), cultured at 37 °C in a humidified atmosphere with 5% CO₂ and passaged every 3–4 days.

IgE antibody preparation

IgE antibody was generated from supernatant of U266B1 cells, which produce IgE antibody. U266B1 cells were incubated without medium exchange for

Figure 1. Enhanced expression of Fc ϵ RI on the cell surface IgE/HC- or IgE/SNP-treated KU812 cells. The expression of Fc ϵ RI on the cell surface of KU812 cells was analyzed by flow cytometry. The cell surface Fc ϵ RI was detected by incubating the cell with mouse anti-human Fc ϵ RI α chain mAb (CRA-1) and subsequent reacting the cells with FITC-conjugated goat anti-mouse immunoglobulins (white). Mouse IgG was used as an isotype-matched control antibody (gray). The percentage of cells expressing the cell surface Fc ϵ RI was shown in each figure. The results are a representative of three independent experiments. A, non-treated KU812 cells; B, KU812 cells treated with 1 μ M HC for 10 days; C, KU812 cells treated with 1 nM SNP for 14 days; D, KU812 cells treated with 450 ng ml $^{-1}$ for 6 days; E, KU812 cells treated with 450 ng ml $^{-1}$ IgE and 1 μ M HC for 8 days; F, KU812 cells treated with 450 ng IgE and 1 nM SNP for 4 days.

7 days, and the supernatant was generated by centrifugation and filtration. The IgE concentration in the supernatant was measured by the enzyme-linked immunosorbent assay (ELISA).

ELISA

A 96 well plate was coated overnight at 4 $^{\circ}$ C with 1 μ g ml $^{-1}$ of anti-human IgE antibody in 100 μ l carbonate buffer (pH 9.6), washed, and blocked by 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS; pH 7.5) at 37 $^{\circ}$ C for 2 h. After washing, samples were diluted with 1% BSA in PBS and incubated at 37 $^{\circ}$ C for 2 h. After washing, captured IgE was detected with 0.5 μ g ml $^{-1}$ of horseradish peroxidase-conjugated goat anti-human IgE, and the plate was thoroughly washed and the color reaction with 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic

acid) (ABTS) was measured at 405 nm using a plate reader.

Flow cytometric analysis of Fc ϵ RI expression

Expression of the cell surface Fc ϵ RI was assessed by indirect immunofluorescence and flow cytometry. In brief, KU812 cells (1×10^6 cells) were incubated with anti-human Fc ϵ RI α chain antibody (10 μ g ml $^{-1}$) for 60 min on ice. Then, the cells were washed with PBS for 60 min on ice and stained with FITC-conjugated goat anti-mouse antibody (20 μ g ml $^{-1}$) for 60 min on ice. The cells were washed with PBS and subjected to flow cytometry (FACSCalibur, Becton Dickinson, San Jose, CA). As a negative control, a mouse IgG antibody (10 μ g ml $^{-1}$), replacing anti-human Fc ϵ RI α chain antibody was used. To detect cell surface CD13 and CD15 antigens, mouse anti-

Figure 2. Activities of transcription factors in the IgE/HC- or IgE/SNP-treated KU812 cells. To evaluate the activities of transcription factors, the binding activities of AP-1, NF-AT and NF- κ B in the IgE/HC- or IgE/SNP-treated KU812 cells were examined by the EMSA analysis. The sequences of probes and the conditions of binding reaction were described in the Materials and methods section in detail. Non-labeled probes were used as competitors for each experiment. The results are a representative of three independent experiments. A, AP-1; B, NF-AT; C, NF- κ B. Lane 1, non-treated KU812 cells (control); lane 2, non-treated KU812 cells + competitor; lane 3, 450 ng ml⁻¹ IgE-treated KU812 cells, 4 days; lane 4, 450 ng ml⁻¹ IgE-treated KU812 cells, 8 days; lane 5, 100 nM HC/450 ng ml⁻¹ IgE-treated KU812 cells, 4 days; lane 6, 1000 nM HC/450 ng ml⁻¹ IgE-treated KU812 cells, 8 days; lane 7, 100 nM HC/450 ng ml⁻¹ IgE-treated KU812 cells, 8 days; lane 8, 1000 nM HC/450 ng ml⁻¹ IgE-treated KU812 cells, 8 days; lane 9, 10 pM SNP/450 ng ml⁻¹ IgE-treated KU812 cells, 4 days; lane 10, 100 pM SNP/450 ng ml⁻¹ IgE-treated KU812 cells, 4 days; lane 11, 1000 pM SNP/450 ng ml⁻¹ IgE-treated KU812 cells, 4 days; lane 12, 10 pM SNP/450 ng ml⁻¹ IgE-treated KU812 cells, 8 days; lane 13, 100 pM SNP/450 ng ml⁻¹ IgE-treated KU812 cells, 8 days; lane 14, 1000 pM SNP/450 ng ml⁻¹ IgE-treated KU812 cells, 8 days.

human CD13 mAb (IgG type, $10 \mu\text{g ml}^{-1}$) and mouse anti-human CD15 mAb (IgM type, $10 \mu\text{g ml}^{-1}$) were used, respectively. For simultaneous staining of CD13 and CD15 antigens, the CD13 antigen was detected by the PE-conjugated goat anti-mouse IgG antibody ($20 \mu\text{g ml}^{-1}$), and the CD15 antigen by the FITC-conjugated goat anti-mouse IgM antibody ($20 \mu\text{g ml}^{-1}$).

Electrophoretic mobility shift assay (EMSA)

Cells (1×10^8 cells) cultured with IgE/Hc or IgE/SNP were harvested and pelleted in 15 ml of PBS by centrifugation for 5 min at $1000 \times g$. Nuclear extracts were prepared according to the Dignam's method with some modifications (Dignam et al., 1983). In brief, the cells were washed with PBS, and resuspended in 1 ml of buffer A (10 mM HEPES, pH 7.6, 15 mM KCl, 2 mM MgCl_2 , 0.1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM DTT, 0.5 mM phenylmethanesulfonyl fluoride (PMSF) and $10 \mu\text{g ml}^{-1}$ leupeptin). After removal of the soluble fraction by centrifugation ($800 \times g$, 1 min) at 4°C , the pellets were resuspended in 1 ml of buffer B (buffer A containing 0.2% NP-40). Furthermore, the pellets were resuspended in 1 ml of sucrose buffer (0.25 M sucrose, 10 mM HEPES, pH 7.6, 15 mM KCl, 2 mM MgCl_2 , 0.1 mM EDTA, 1 mM DTT, 0.5 mM PMSF and $10 \mu\text{g ml}^{-1}$ leupeptin), centrifuged, and resuspended in 20 μl of buffer D (50 mM HEPES, pH 7.9, 400 mM KCl, 0.1 mM EDTA, 10% glycerol, 1 mM DTT, 0.5 mM PMSF, and $10 \mu\text{g ml}^{-1}$ leupeptin). The cell suspensions were incubated for 30 min at 4°C with agitation and centrifuged at $600 \times g$ for 15 min at 4°C . The supernatant was applied to EMSA. Protein concentrations were measured according to the method described by Bradford with a commercial reagent (Bradford, 1976). Double strand oligonucleotides (40 pmol) containing the NF- κB , NF-AT and AP-1 consensus sequences were end-labelled [γ - ^{32}P] dATP and used as a probe in EMSA. Sequences of sense strands of these oligonucleotides were as follows; NF- κB (sense) 5'-AGTTGAGGGGACTTCCCAGGC-3', NF- κB (antisense) 5'-GCCTGGGAAAGTCCCC-TCAACT-3', NF-AT: (sense) 5'-GATCCGGAGGAA-AACTGTTTCATACAGAAGGCGTG-3', NF- κB (antisense) 5'-GATCCACGCCTTCTGTATGAAAC-AGTTTTTCCTCCG-3' and AP-1 (sense) 5'-GATCCGGTTGCTGACTAATTG-3' AP-1 (antisense) 5'-GATCCAATTAGTCAGCAACCG-3'. The binding reactions (in total 10 μl) were done by an incubation

of 0.8 μg of the nuclear extract with a [^{32}P]-labeled double-strand oligonucleotide probe (1 pmol) in the reaction buffer (10 mM Tris-HCl, pH 7.5, 10 mM KCl, 1 mM DTT, 1 mM EDTA, 12.5% glycerol, 0.1% Triton X-100, $250 \mu\text{g ml}^{-1}$ BSA, $50 \mu\text{g ml}^{-1}$ poly (dI-dC) poly (dI-dC)) in the presence or absence of a competitor for 15 min at room temperature. The resulting DNA-protein complexes were resolved in 5% polyacrylamide gel electrophoresis. Bands were visualized by autoradiography.

Allergic patient's serum preparation

An allergic patient's serum was generated, and the IgE levels were measured by ELISA. Blood was taken into heparin-untreated tubes, and kept at 37°C for 30–60 min to make the clot. The blood taken off the clot was kept at 4°C overnight, and centrifuged at $10\,000 \times g$ for 10 min at 4°C . The supernatant, which is serum, was collected in a new tube and glycerol was added to the final concentration of 50%. Then, the serum was sterilized and stored at -20°C . Antigen-specificity of the serum against various allergens, such as mite, rice, ovalbumin (OVA) and β -lactoglobulin (β -LG), was determined by ELISA.

Histamine release

The KU812F cells (3×10^6 cells), which were differentiated with the $2.5 \mu\text{g ml}^{-1}$ IgE and 1 μM Hc, were resuspended in 200 μl of 0.1% BSA in PBS, and stimulated with human myeloma IgE ($10 \mu\text{g ml}^{-1}$) and anti-human IgE antibody ($5 \mu\text{g ml}^{-1}$) at 37°C for 30 min. The allergic patient's IgE and a mite allergen were used in $20 \mu\text{g ml}^{-1}$ and $10 \mu\text{g ml}^{-1}$, respectively. Supernatants were collected after centrifugation ($900 \times g$, 5 min). To determine intracellular total histamine, differentiated cells were resuspended with 500 μl of PBS containing 0.1% BSA and sonicated on ice for 1 min, and the supernatants were collected by centrifugation ($10\,000 \times g$, 5 min). The amount of histamine in the supernatant was measured by high performance liquid chromatography (HPLC). The HPLC conditions were as follows; column: HistaminePak (I.D. 6.0 mm $\times$ 50 mm L); detector: Waters Type 470; wavelength: Ex 345 nm, Em 455 nm; column temperature: 70°C ; flow rate: 0.6 ml/min; injection volume: 20 μl ; eluent: sodium citrate buffer (pH 10.0) (11.7 g sodium citrate, 2.0 g of boric acid in 940 ml of distilled water); reaction solution: *o*-phthalaldehyde (OPA) solution (24.7 g boric acid, 23.0 g KCl, 2.0 ml 2-mercaptoethanol, 2.3 ml 30%

Figure 3. Histamine release in human IgE and anti-human IgE-sensitized KU812F cells. KU812F cells were differentiated by treatment with $2.5 \mu\text{g ml}^{-1}$ IgE/ $1 \mu\text{M}$ HC for 7 days. The differentiated KU812F cells were stimulated with human IgE ($10 \mu\text{g ml}^{-1}$) and anti-human IgE antibody ($5 \mu\text{g ml}^{-1}$) for 30 min, then the amount of released histamine was measured by HPLC. The results are a representative of three independent experiments.

Brij-35 solution and 1.6 g OPA in 970 ml distilled water).

Results and discussion

The combined use of HC and SNP with IgE enhances the cell surface FcεRI expression in KU812 cells

KU812 cells are an immature basophilic cell line expressing a low level of high-affinity receptor for IgE. This suggests that additional signals are required to enhance the expression of FcεRI on the cell surface. Recent studies reported that HC and SNP could induce the expression of FcεRI on the cell surface (Hara et al., 2000; Yamashita et al., 2001). Furthermore, it is known that IgE enhances the FcεRI expression on mast cell (Lantz et al., 1997; Yamaguchi et al., 1997, 1999). Based on these studies, we examined whether the expression of FcεRI was enhanced by the treatment of KU812 cells with IgE/HC or IgE/SNP. As

shown in Figure 1, about 11% of non-treated KU812 cells expressed FcεRI on the cell surface (Figure 1A). About 31% of the cell surface FcεRI expression was observed in HC- and SNP-treated KU812 cells (Figures 1B and C). Similarly, the cells treated with 450 ng ml^{-1} IgE for 6 days also expressed 37% FcεRI on their surface (Figure 1D). On the other hand, KU812 cells treated with both 450 ng ml^{-1} IgE and $1 \mu\text{M}$ HC for 8 days expressed about 54% FcεRI on their surface (Figure 1E). KU812 cells treated with both 1 nM SNP and 450 ng ml^{-1} IgE for 4 days expressed 33% FcεRI (Figure 1F). These results suggest that the combination of IgE and HC is most effective for the induction of the cell surface FcεRI of KU812 cells.

Analysis of CD13 and CD15 antigens on the surface in KU812 cells

Basophils and eosinophils can be discriminated from each other by their surface expression of CD anti-

Figure 4. Evaluation of antigen-specific histamine release in the allergic patient's IgE- and mite allergen-sensitized KU812F cells. (A) Antigen-specificity of allergic patient's serum was assessed by ELISA. (B) Differentiated KU812F cells were stimulated with allergic patient's IgE ($20 \mu\text{g ml}^{-1}$) and mite allergen ($10 \mu\text{g ml}^{-1}$) for 30 min, and then the amount of released histamine was measured by HPLC. The total amount of intracellular histamine in differentiated KU812F was also determined after sonication. The results are a representative of three independent experiments.

gens. Although basophils express the CD13 antigen, but not the CD15 antigen, whereas eosinophils express both of the CD antigens. Based on this criterion, we characterized HC-, SNP-, IgE-, IgE/HC- and IgE/SNP-treated KU812 cells by flow cytometric analysis. Table 1 showed that IgE- or IgE/HC-treated KU812 cells maintained almost the same cell population of CD13⁺ and CD15⁻ cells similar to that of non-treated KU812 cell, namely about 95% of cells were CD13⁺/CD15⁻ cells. On the other hand, KU812 cells treated with SNP differentiated into eosinophils, which was demonstrated by the shift of the cell population from CD13⁺/CD15⁻ to CD13⁺/CD15⁺ cell population (Yamashita et al., 2001). However, most of the IgE/SNP-treated KU812 cells expressed the CD13 antigen, but not the CD15 antigen (Table 1). These results suggested that IgE induced basophil-like cell surface expression of the CD antigens, even in the presence of SNP.

Signals transmitted in IgE/HC- or IgE/SNP-treated KU812 cells

We expected that extracellular factors caused the bi-directional cell differentiation of KU812 cells via the intracellular signal transduction pathways. Thus we analyzed the activation of several transcription factors concerning the activation of immune cells by the EMSA. NF- κ B and AP-1 are known to be activated upon Fc ϵ RI cross-linking and subsequent protein kinase C activation, and NF-AT is activated in response to the increase in cellular calcium concentration following the activation of Fc ϵ RI (Schroeder and MacGlasham, 1997). As shown in Figure 2, IgE activated AP-1 and NF-AT but not NF- κ B. It was reported that these transcription factors were not activated in non-treated and SNP-treated KU812 cells (Yamashita et al., 2001). However, the combined use of HC or SNP with IgE resulted in activation of the activities of AP-1, NF-AT and NF- κ B, depending upon the conditions (Figure 2). These results suggest that IgE/HC or IgE/SNP transmit signals intracellularly and activates these transcription factors, resulting in induction of basophilic-like gene expression and basophilic differentiation. Yamashita et al. (2001) reported that SNP-treated KU812 cells expressed mRNA of eosinophil-derived neurotoxin (EDN) and eosinophil peroxidase (EPO), which were markers of differentiation into eosinophils. We suggested that KU812 cells were multi-potent precursor cells, which could differ-

entiate into basophils and eosinophils depending upon exogenous signals.

Histamine release from IgE and anti-IgE sensitized-KU812F cells

It is well recognized that degranulation of basophils and mast cells in allergic reaction is triggered upon aggregation of the Fc ϵ RI which is bound to allergen-specific IgE on the cell surface (Metzger, 1991; Taudou et al., 1993). We assessed whether histamine release from KU812 cells treated with IgE and HC can be elicited upon stimulation with human IgE/anti-IgE mAb. KU812F cells were cultured with both 2.5 μ g ml⁻¹ IgE and 1 μ M HC for 7 days, and stimulated for degranulation with human IgE/anti-IgE mAb. The KU812F cells cultured in the presence of IgE and HC released histamine when the cells were stimulated with IgE/anti-IgE mAb (Figure 3), suggesting that this system could be applied to construct a practical *in vitro* allergy reaction evaluation system for general use.

Histamine release from allergic patient's IgE and mite allergen-sensitized KU812F cells

We attempted to evaluate an allergic patient's serum and specific allergen using the constructed *in vitro* allergy reaction evaluation system using differentiated KU812F cells. Firstly antigen-specificity of an allergic patient's serum was evaluated by ELISA. As shown in Figure 4A, the allergic patient's IgE was indicated to have a strong reactivity against mite allergen. The differentiated KU812F cells, which were induced the cell surface Fc ϵ RI by the treatment with both IgE and HC, were stimulated with the allergic patient's IgE and mite allergen for degranulation. As the result, histamine release was observed (Figure 4B). These results indicate that a practical *in vitro* allergy reaction evaluation system for general use was constructed using IgE/HC-treated KU812F cells, overcoming the unstable differentiated phenotypes of KU812F cells. This system will be useful to analyze allergic reaction and functions of bioactive substances in allergic diseases.

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