Role of mitogen-activated protein kinase and phosphoinositide 3-kinase in the differentiation of rat pancreatic AR42J cells induced by hepatocyte growth factor

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Abstract

Aims/hypothesis. Pancreatic AR42J cells express both exocrine and neuroendocrine properties. When exposed to activin A, approximately 50 % of the cells die within 3 days by apoptosis. Addition of hepatocyte growth factor prevents apoptosis induced by activin A and induces differentiation into insulin-producing cells. The present study was conducted to examine the role of mitogen-activated protein kinase and phosphoinositide 3-kinase in the action of hepatocyte growth factor.

Methods. The role of mitogen-activated protein kinase was assessed by using 2-(2'-amino-3'-methoxyphenyl)-oxanaphthalen-4-one (PD098059). Cells were also transfected with cDNA for mitogen-activated protein kinase phosphatase and constitutively active mutant of mitogen-activated protein kinase kinase.

Results. Hepatocyte growth factor induced sustained activation of the mitogen-activated protein kinase, which was inhibited by PD098059. PD098059 com-

pletely blocked the differentiation and also blocked the prevention of apoptosis. Transfection of the cells with cDNA for mitogen-activated protein kinase phosphatase reproduced the effect of PD098059. Conversely, transfection with cDNA for the constitutively active mutant of mitogen-activated protein kinase kinase reproduced the effect of hepatocyte growth factor. In contrast, addition of wortmannin or transfection of the dominantly negative form of the p85 subunit of the phosphoinositide 3-kinase did not affect differentiation induced by hepatocyte growth factor. Instead, wortmannin enhanced the increase in the insulin content of the differentiated AR42J cells.

Conclusion/interpretation. The MAP kinase pathway is necessary and sufficient for the action of HGF on differentiation of AR42J cells. [Diabetologia (1999) 42: 450–456]

Keywords Insulin, beta cell, differentiation, hepatocyte growth factor, MAP kinase.

Pancreatic endocrine and exocrine cells are thought to arise from the common precursor cells during development [1, 2]. Neogenesis of endocrine cells from the

progenitor in the pancreatic duct also occurs after the destruction of pancreatic beta cells in adult animals. The precise differentiation process and the mechanism regulating the differentiation of pancreatic beta cells are of considerable interest. The formation of pancreatic endocrine cells is presumed to be determined by sequential activation of various transcription factors and several transcription factors regulating differentiation of pancreatic endocrine cells have been postulated [3–6]. It is also conceivable that various extracellular signalling molecules regulate the formation and differentiation of pancreatic endocrine cells. Thus, various growth and differentiation factors are thought to be involved in the formation, proliferation and differentiation of pancreatic endocrine cells.

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Abbreviations: HGF, Hepatocyte growth factor; MAP, mitogen-activated protein; PI, phosphoinositide; ICC, islet cell-like cluster; MAPKK, mitogen-activated protein kinase kinase; GFP, green fluorescent protein; PD098059, 2-(2'-amino-3'-methoxyphenyl)-oxanaphthalen-4-one; LY294002, 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one.

Pancreatic AR42J cells are derived from rat pancreatic acinar tumours and possess both exocrine and neuroendocrine properties [7]. Upon treatment with activin A, AR42J cells differentiate into cells with neuron-like morphology [8]. In addition to the morphological changes, activin treatment leads to the expression of the ATP-sensitive potassium channel and furthermore, the expression of a pancreatic hormone, pancreatic polypeptide [8, 9]. Hence, activin A converts amylase-secreting AR42J cells into endocrine cells. Activin-treated AR42J cells further differentiate into insulin-producing cells by treatment with either betacellulin [9] or hepatocyte growth factor (HGF) [10]. Taken together, AR42J cells could be a useful model system to study the formation of insulin-producing cells from the common precursor cells.

Hepatocyte growth factor (HGF) is a multifunctional factor that modulates growth, differentiation, motility, and organogenesis [11, 12]. It is synthesized in mesenchymal cells and has been shown to increase the number of insulin-producing cells in cultured human islet cell-like clusters (ICC) [13]. It also mediates beta-cell mitogenic signals from the fetal mesenchyme to the developing beta cell [14] and is possibly one of physiological regulators of differentiation of pancreatic endocrine cells. In this study, we investigated the signal transduction pathways responsible for the formation and maturation of insulin-producing cells from AR42J cells induced by HGF.

Materials and methods

Materials. Recombinant human HGF was purchased from R and D Systems (Minneapolis, Minn., USA) and epidermal growth factor (EGF) was from Collaborative Research (Lexington, Mass., USA). Recombinant human activin A was supplied by Dr. Y. Eto of Central Research Laboratory, Ajinomoto (Kawasaki, Japan). The 2-(2'-amino-3'-methoxyphenol)-oxanaphthalen-4-one (PD098059), which inhibits the activation of mitogen activated protein kinase kinase (MAPKK) by Raf-1 [15], was provided by Dr. A. Saltiel of Parke-Davis (Ann Arbor, Mich., USA). Wortmannin was from Sigma Chemicals (St. Louis, Mo., USA) and was dissolved in dimethyl sulfoxide at a concentration of 10 mmol/l. The 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002) was from Calbiochem (La Jolla, Calif., USA). The materials required for the assay of mitogen-activated protein (MAP) kinase activity (including specific peptide substrate) and [γ - 32 P]ATP were provided by Amersham Pharmacia Biotech, (Amersham Bucks, UK). Dr. E. Nishida of Kyoto University (Kyoto, Japan) provided cDNAs encoding MAP kinase phosphatase and constitutively active mutant of MAPKK. Dr. M. Kasuga of Kobe University (Kobe, Japan) provided cDNA encoding dominantly negative mutant of p85 subunit of PI 3-kinase.

Cell culture. We used AR42J-B13 cells, a subclone of AR42J, which convert into insulin-positive cells in response to HGF and activin A [10]. These cells were maintained in DMEM containing 20 mmol/l HEPES/NaOH (pH 7.4), 5 mmol/l NaHCO₃, penicillin, streptomycin and 10% FCS at 37°C under a humidified condition of 95% air and 5% CO₂. The number of viable

cells was counted after staining the cells with acridine orange/ethidium bromide [16]. This method makes non-viable cells visible but does not discriminate apoptotic cells from necrotic cells.

Measurement of MAP kinase activity. We seeded AR42J-B13 cells at a density of 2×10^5 cells/ml in 6-well dishes (Falcon, Becton Dickinson, Lincoln Park, N.J., USA). Cells were serum-starved for 24 h and then incubated with HGF, EGF, insulin or activin A for the time indicated at 37°C. To examine the effects of PD098059, cells were pretreated with the compound for 60 min and then HGF was added. The cells were further incubated for various times. After the treatment, cells were harvested by scraping in 400 μ l of lysis buffer containing 10 mmol/l TRIS/HCl (pH 7.4), 150 mmol/l NaCl, 2 mmol/l phenylmethylsulphonyl fluoride, 10 μ g/ml leupeptin and 10 μ g/ml aprotinin. Cellular debris were precipitated by centrifugation at $25,000 \times g$ for 20 min and the supernatant was retained to obtain cytoplasmic MAP kinase. Samples (15 μ l) and 10 μ l of the substrate buffer containing 6 mmol/l specific substrate were added to each tube together with 5 μ l of [γ - 32 P]ATP (37 KBq (1.0 μ Ci) of [γ - 32 P]ATP) and 9 mmol/l MgCl₂ and incubated for 30 min in a water bath at 30°C. The reaction was terminated by adding 10 μ l of 300 mmol/l orthophosphoric acid. From each sample 30 μ l was spotted onto a phosphocellulose disc, washed three times in 0.5% phosphoric acid for 30 min and once in distilled water for 5 min. The radioactivity on each disc was then determined by scintillation counting [17].

Measurement of phosphoinositide 3-kinase activity. Cells were incubated for various times with HGF in the presence and absence of wortmannin. The cells were rinsed with an ice-cold buffer containing 20 mmol/l HEPES/NaOH (pH 7.5), 150 mmol/l NaCl, and 0.1 mmol/l Na₃VO₄ and then lysed in 300 μ l of this buffer supplemented with 10% glycerol, 1% Nonidet P-40 (Sigma Chemicals), 5 mmol/l EDTA, 1 mmol/l phenylmethylsulphonyl fluoride, 10 μ g/ml leupeptin and 10 μ g/ml pepstatin A. The cell lysates were centrifuged at $25,000 \times g$ for 20 min and the supernatant was immunoprecipitated with an anti-phosphotyrosine antibody coupled to protein G-sepharose. Phosphoinositide (PI) 3-kinase activity in the immunoprecipitate was measured as described previously [18].

Transfection of cDNA. We seeded AR42J-B13 cells on non-coated glass cover slips and cultured them for 20 h before transfection. Then 2 μ g/well of the transient expression vector containing SV40 promoter encoding constitutively active mutant of MAP kinase kinase (pcDL-SR α 456, 32–51 S218E S222E MAPKK) and of encoding MAP kinase phosphatase (SR α 456-CL100) were transfected with a liposome method using LIPOFECTAMINE Regent (Gibco, Gland Island, N. Y., USA). Cells were co-transfected with 0.2 μ g/well plasmid pGL-1 containing cytomegalo-virus promoter encoding green fluorescent protein (GFP) (Gibco) [19] to effectively monitor transfected cells. Efficiency of transfection was 15–30%.

Antibodies. Polyclonal antibody against insulin was provided by Dr. K. Wakabayashi of the Institute for Molecular and Cellular Regulation, Gunma University (Maebashi, Japan). As a second antibody, we used indocarbocyanine-conjugated donkey anti-guinea pig IgG (Cappel, Turnhout, Belgium) [9].

Immunofluorescence microscopy. Cells were cultured on non-coated glass coverslips. To examine the effects of PD098059 or wortmannin on producing insulin-positive cells, AR42J-B13 cells were pretreated with the compound for 60 min. The cells were then cultured with 100 pmol/l of HGF and 4 nmol/l activin A for 72 h with and without PD098059 or wortmannin.

Fig. 1A, B. Effect of HGF on the activity of MAP kinase. **A** AR42J-B13 cells were incubated for indicated time with (●) or without (○) 100 pmol/l HGF or 1 nmol/l EGF (△) and the activity of MAP kinase was measured as described in the Methods. Values are the means $\pm$ SD for four experiments each done in duplicate. **B** AR42J-B13 cells were incubated for 60 min with 100 pmol/l HGF and various concentrations of PD098059. The MAP kinase activity was then measured. Values are the means $\pm$ SD for four experiments

Cells transfected with cDNA for constitutively active mutant of MAP kinase kinase were cultured with or without 4 nmol/l activin A and cells transfected with cDNA for MAP kinase phosphatase were cultured with 100 pmol/l HGF and 4 nmol/l activin A for 24 h. The cells were fixed with 3% paraformaldehyde in PBS for 30 min, permeabilized with 0.1% (vol/vol) Triton X-100 in PBS for 5 min and incubated sequentially with Block Ace (Morinaga, Tokyo, Japan) for blocking, the first antibody (anti-insulin; 1:500) and the second antibody (1:1500). The cells were examined with an Olympus BX50 microscope equipped with a BX-fluorescence attachment and dual-band filter (U-MFI/TRITC) (Olympus Optical, Tokyo, Japan). For measurement of insulin-positive cells, incubation was in quadruplicate and 500 cells were counted in each dish.

Measurement of insulin content. Cells were incubated with activin A and HGF for 72 h. Then the cells were washed with DMEM and were further incubated with HGF for 5 days in the presence and absence of wortmannin. Cells were lysed by sonication and insulin was extracted using 0.1 mol/l HCl. Insulin content of the cells was measured by using a time-dissolved immunofluorometric assay system [9]. The statistical significance was determined by Student's *t* test.

Reverse transcription-PCR. We extracted mRNA by using the Quick Prep (Pharmacia, Piscataway, N.J., USA). Samples of mRNA were treated with DNase to remove any trace of contamination by genomic DNA. First-stranded cDNA was synthesized by using a preamplification system for First Stand cDNA Synthesis Kit (Gibco). To confirm that there was no contamination of genomic DNA, samples without reverse-transcriptase treatment were prepared. We carried out PCR as described previously [9].

Results

Effect of HGF on MAP kinase and PI 3-kinase activities. Hepatocyte growth factor activates various intracellular signalling pathways. In this study, we investigated the role of MAP kinase and PI 3-kinase in the action of HGF on differentiation of AR42J cells. We first examined the effects of HGF on the activities of MAP kinase and PI 3-kinase. We found, 100 pmol/l HGF increased the MAP kinase activity in AR42J cells (Fig. 1A). The effect of HGF was biphasic. The MAP kinase activity was at a maximum at 60 min, reached the plateau level at 90 min and remained raised for at least 180 min (Fig. 1A). The effect of HGF was sustained but the effects of other growth factors, for example EGF and insulin (data not shown) were only transient although the peak value was nearly identical. The MAP kinase activity after EGF or insulin peaked at 30 min but returned to the basal level by 60 min. AR42J cells differentiate into insulin-producing cells by a combination of HGF and activin A [10]. Activin A did not affect the MAP kinase activity by itself or alter the effect of HGF (data not shown). The activation of MAP kinase kinase induced by Raf-1 is inhibited by PD098059 [15]. We found PD098059 inhibited HGF-induced activation of the MAP kinase in a concentration-dependent manner (Fig. 1B). At 100 μ mol/l, PD098059 nearly completely blocked the activity of MAP kinase.

A rapid activation of PI 3-kinase was induced by HGF (Fig. 2). Wortmannin is a compound which blocks the activity of PI 3-kinase at concentrations around 100 nmol/l [20]. We found 100 nmol/l wortmannin completely inhibited the PI 3-kinase activated by HGF in AR42J cells (Fig. 2).

Effect of PD098059 and wortmannin on the formation of insulin-producing cells. To assess the involvement of MAP kinase or PI 3-kinase or both in HGF-induced differentiation, we examined the effects of PD098059 and wortmannin on the formation of insulin-producing cells induced by a combination of HGF and activin A. After treatment with activin A and HGF for 72 h AR42J cells convert to insulin-producing cells [10]. When cells were incubated for 72 h with activin A alone, the cells began to synthesize pancreatic polypeptide [8, 9]. The morphology of the cells changed considerably [8] but we found the num-

Fig. 2 A, B. Effect of HGF on the activity of PI 3-kinase. **A** AR42J-B13 cells were incubated for 5 min with 100 pmol/l HGF and the activity of PI 3-kinase was measured as described in the Methods. The result is representative of three experiments. **B** AR42J-B13 cells were incubated with 100 pmol/l HGF in the presence and absence of 100 nmol/l wortmannin and the activity of PI 3-kinase was measured. The result is representative of two experiments

number of living cells were greatly reduced (Fig. 3). Activin A induced apoptosis since DNA ladder formation was observed in cells treated with activin A (data not shown). Addition of HGF blocked the reduction of the number of living cells induced by activin A (Fig. 3) and further induced differentiation into cells expressing insulin. Most of the AR42J cells treated with HGF and activin A for 72 h became insulin-positive. Addition of 50 μ mol/l PD098059 blocked the formation of insulin-producing cells. At this concentration of PD098059, the number of living cells was slightly reduced. There was a dose-response relation for PD098059-induced inhibition of the effect of HGF (Fig. 4). Incubation of AR42J cells with a combination of activin A- and HGF-induced differentiation in about 80 % of the cells (Fig. 4). PD098059 inhibited HGF-induced differentiation in a concentration-dependent manner. It also inhibited the expression of mRNA for insulin induced by HGF (Fig. 5).

Fig. 3. Effect of activin A and HGF on the viability of AR42J cells. AR42J-B13 cells were incubated with 4 nmol/l activin A in the presence and absence of 100 pmol/l HGF for 3 days and the number of living cells was counted. In some experiments, 100 μ mol/l PD098059 or 100 nmol/l wortmannin was added. Values are the means $\pm$ SD for four experiments. * $P < 0.01$ vs none, ** $P < 0.05$ vs none

As noted above, 100 μ mol/l PD098059 completely blocked HGF-induced differentiation but at this concentration, the number of living cells was reduced by approximately 50 % (Fig. 3).

In contrast, AR42J cells converted to insulin-positive cells even in the presence of 100 nmol/l wortmannin (data not shown) and mRNA for insulin was detected in the presence of wortmannin (Fig. 5). Since wortmannin is relatively unstable, we added it every 24 h in some experiments, but the results were the same. Furthermore, similar results were obtained by using LY294002, another inhibitor of PI 3-kinase (data not shown). Note that wortmannin reduced the number of living cells (Fig. 3).

Effect of transfection of cDNA for MAP kinase phosphatase and constitutively active mutant of MAP kinase kinase. To confirm the involvement of MAP kinase in the HGF-induced differentiation, we transfected cells with cDNA for MAP kinase phosphatase, which blocks the activation of the MAP kinase [21]. We transfected AR42J cells with cDNA for MAP kinase phosphatase together with that for GFP. Cells that effectively transfected were identified by green fluorescence of GFP. Both transfected and untransfected cells were incubated for 24 h with a combination of HGF and activin A. Transfected cells expressing GFP did not express immunoreactive insulin whereas cells not transfected effectively expressed insulin (Fig. 6). Immunoreactivity of insulin disappeared in all the cells effectively transfected with GFP (Table 1).

We also transfected AR42J cells with cDNA for constitutively active mutant of MAPKK, which activates MAP kinase [22]. Cells were transfected with

Fig. 4. Dose-response relation for PD098059-induced inhibition of differentiation. AR42J-B13 cells were incubated for 3 days with 100 pmol/l HGF and 4 nmol/l activin A in the presence of various concentrations of PD098059. The number of insulin-producing cells was examined by immunocytochemistry. Values are the means $\pm$ SD for four experiments

Fig. 5. Expression of mRNA for insulin. AR42J-B13 cells were incubated for 3 days with 100 pmol/l HGF and 4 nmol/l activin A in the absence and presence of 100 μmol/l PD098059 or 100 nmol/l wortmannin. RNA was extracted and mRNA for insulin was measured by RT-PCR

cDNA for constitutively active mutant of MAPKK together with cDNA for GFP. Both transfected and untransfected cells were incubated for 24 h with activin A and effectively transfected cells were identified by fluorescence of GFP. Effectively transfected cells incubated with activin A became insulin-positive (Fig. 6). Immunoreactivity of insulin was detected in more than 80 % of the cells expressing GFP (Table 1). Transfected cells did not become insulin-positive in the absence of activin A treatment (data not shown). To confirm the absence of an involvement of PI 3-kinase in the action of HGF on differentiation, we introduced cDNA for dominantly negative mutant of p85 subunit of the PI 3-kinase [23] together with that for GFP. Cells transfected with the dominantly negative p85 converted to insulin-positive cells when incubated with HGF and activin A (Table 1).

Blockers of PI 3-kinase have been reported to enhance the differentiation of ICC [24]. We therefore further studied whether wortmannin altered the effect of HGF. We found AR42J cells converted to insulin-producing cells after the treatment with activin A and HGF for 72 h. Once differentiated, however,

Fig. 6A, B. Effect of transfection of MAP kinase phosphatase and constitutively active MAPKK on differentiation. **A** AR42J-B13 cells were co-transfected with cDNA for MAP kinase phosphatase and GFP. The cells were then incubated with 100 pmol/l HGF and 4 nmol/l activin A for 24 h. The insulin-producing cells were then examined by immunocytochemistry. The triangular cell expressing GFP (green color) did not express insulin whereas the spindly cell not expressing GFP expressed insulin (orange color) at the end of the processes. **B** AR42J-B13 cells were co-transfected with cDNA for the constitutively active mutant of MAP kinase kinase and GFP. The cells were then incubated with 4 nmol/l activin A for 24 h and the insulin-producing cells were then examined. The cells expressing GFP also expressed insulin

these cells survived only a few days in the presence of activin A and HGF. When differentiated cells were then incubated with HGF alone, they survived for more than 7 days. In AR42J cells, HGF is not a mitogen [10]. In agreement with this notion, HGF did not increase the number of the cells under this condition. Instead, insulin content of the cells increased (Fig. 7). Addition of wortmannin further increased the insulin content of the cells.

Discussion

Both exocrine and neuroendocrine properties are expressed by AR42J cells [7]. Upon treatment with a combination of activin A and HGF, AR42J cells differentiate into insulin-secreting cells within 3 days [10]. Although AR42J cells are tumour cells, they can differentiate into insulin-secreting cells via a programmed scenario. Being a clonal cell line, they provide a good experimental system to study the differentiation of beta cells, in particular, to study the signal transduction system responsible for the action of

Table 1. Effect of transfection of cDNA for MAP kinase phosphatase, constitutively active MAPKK and dominantly negative PI 3-kinase on the differentiation of AR42J cells

Transfected genes	Cell number (%)			
	Cells studied	Positive for GFP alone	Positive for insulin alone	Double-positive
MAP kinase phosphatase	980 (100)	245 (25.0)	499 (50.9)	0 (0)
Constitutively active MAPKK	955 (100)	33 (3.5)	0 (0)	143 (14.9)
Dominantly negative PI3K	932 (100)	98 (10.5)	476 (51.0)	136 (14.5)

AR42J cells were transfected with cDNA for MAP kinase phosphatase, constitutively active MAPKK or dominantly negative p85 of the PI 3-kinase. Cells transfected with MAP kinase phosphatase and dominantly negative p85 were incubated with 100 pmol/l HGF and 4 nmol/l activin A for 24 h. Cells

transfected with constitutively active MAPKK were incubated with 4 nmol/l activin A alone for 24 h. Cells were then immunostained with anti-insulin antibody. The number of cells positive for GFP alone, insulin alone and both were counted

Fig. 7. Insulin content of the cells treated with HGF. AR42J-B13 cells were incubated for 3 days with 100 pmol/l HGF and 4 nmol/l activin A. Cells were then washed twice with DMEM and were then incubated for 5 days in DMEM containing 0.5% FCS with 100 pmol/l HGF in the presence or absence of 50 nmol/l wortmannin. As a control, cells pretreated with activin A and HGF were further incubated for 5 days in DMEM containing 0.5% FCS. The insulin content of the cells was then measured. Values are the means $\pm$ SD for four experiments. * $P < 0.05$ vs none, ** $P < 0.05$ vs HGF alone

differentiation factors. In our study, we examined the signal transduction pathways involved in the HGF-mediated differentiation. In AR42J cells, HGF elicited three different actions: firstly, it blocked apoptosis induced by activin A; secondly, it converted AR42J cells into insulin-secreting cells; and thirdly, it increased the insulin content of the AR42J cells already committed to differentiation.

Hepatocyte growth factor activated both MAP kinase and PI 3-kinase. When PD098059 or wortmannin was added together with HGF, only PD098059 blocked the differentiation. Since inhibition of MAP kinase activity correlates well with the attenuation of differentiation, it is probable that the MAP kinase activation is necessary for the HGF-induced differentiation. Consistent with this notion, introduction of the cDNA for MAP kinase phosphatase, which inactivates MAP kinase [21], blocked HGF-induced differentiation. Furthermore, transfection of cDNA for constitutively active mutant of MAPKK led to differ-

entiation. Taken together, these results indicate that activation of MAP kinase pathway is necessary and sufficient for HGF action on differentiation of AR42J cells into insulin-secreting cells. These results corroborate earlier reports that activation of the MAP kinase is necessary and sufficient for differentiation of PC12 cells by nerve growth factor [25, 26]. In AR42J cells, other ligands which did not induce differentiation also activated MAP kinase, for example, EGF- and insulin-activated MAP kinase. One difference between the actions of HGF and these ligands is that the latter induced only a transient activation of the MAP kinase. The EGF-induced activation of MAP kinase peaked at 30 min but returned to the basal value by 60 min whereas HGF activated the MAP kinase for at least 3 h. In PC12 cells, nerve growth factor induces sustained activation of the MAP kinase whereas EGF, which does not induce differentiation in these cells, activates the MAP kinase only transiently [27]. Hence, sustained activation of the MAP kinase is possibly a prerequisite for the induction of differentiation. Alternately, HGF but not EGF may induce translocation of the MAP kinase into the nucleus as shown in other cells [28]. Activin-induced apoptosis in AR42J cells is reversed by HGF (Fig. 3). When 100 μ mol/l PD098059, which completely blocks MAP kinase activation by HGF, was added together with HGF and activin A, the viability of AR42J reduced considerably. This result indicates that the HGF action on the prevention of apoptosis requires the activation of MAP kinase. Hence, MAP kinase mediates the protection of apoptosis and induction of differentiation into insulin-secreting cells. Note that the activation of PI 3-kinase is possibly also required for the HGF action on the prevention of apoptosis. In our system, HGF did not increase the activities of other members of the MAP kinase, for example c-Jun aminoterminal kinase or p38 (data not shown).

In AR42J cells, blocking the PI 3-kinase did not inhibit HGF-induced conversion into insulin-secreting cells. This notion is supported by two means: wortmannin which blocks PI 3-kinase effectively [20] did not block HGF-induced differentiation and transfection of cDNA for the dominantly negative mutant of

p85 subunit of the PI 3-kinase [23] did not block the HGF action. Therefore, HGF induces differentiation of AR42J cells by a mechanism independent of PI 3-kinase, whereas, inhibition of PI 3-kinase activity enhances the effect of HGF on insulin content of already differentiated AR42J cells. It has been reported that in human ICC, blockers of the PI 3-kinase enhanced differentiation of ICC into insulin-secreting cell [24]. That report showed that PI 3-kinase inhibitors increased the number of insulin-positive cells and the insulin content of ICC. The apparent difference in the results of the two studies could result from the difference in the properties of two cell systems, i.e. primary cells compared with tumour cells. Alternately, AR42J cells and ICC could represent different steps of differentiation. The second may consist of mixed cell populations and even though most of the cells are negative for insulin, they are probably already committed to differentiation into endocrine lineage. In contrast, AR42J cells are not committed to differentiation into endocrine cells unless activin A is added exogenously [8, 9]. Before treatment with activin A, AR42J cells express amylase and carbonic anhydrase, markers of acinar and ductal cells, respectively. Possibly AR42J cells are a model in which we can observe an early step of endocrine determination. Once committed to differentiation into insulin-producing cells, PI 3-kinase activity is inhibitory for the maturation of differentiated AR42J cells. If this is the case, our results are in agreement with the earlier results [24].

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