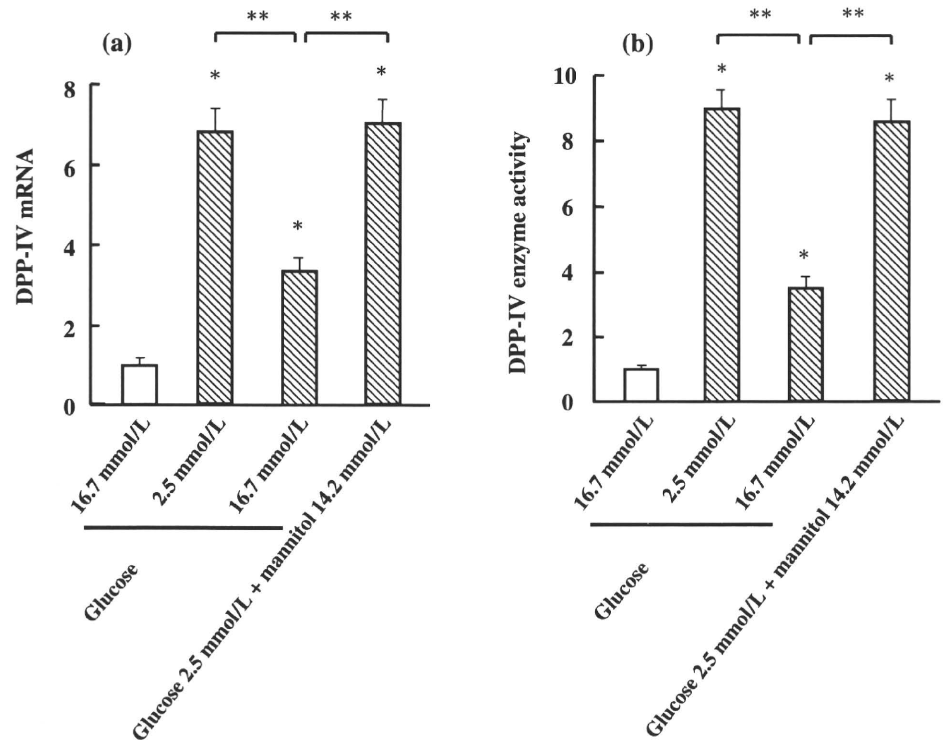

Fig. 1 Dipeptidyl peptidase IV (DPP-IV) gene expression (a) and enzyme activity (b) in Caco-2 cells cultured in the presence of 2.5 or 16.7 mmol/L glucose on Day 1 (□) or 14 (▨) of confluence. Data are the mean±SEM from six independent experiments. *$P < 0.01$ compared with 16.7 mmol/L glucose on Day 1 of confluence. **$P < 0.01$. The DPP-IV gene expression and enzyme activity were normalized against that of cells cultured in the presence of 16.7 mmol/L glucose on Day 1 of confluence, which was defined as one unit.

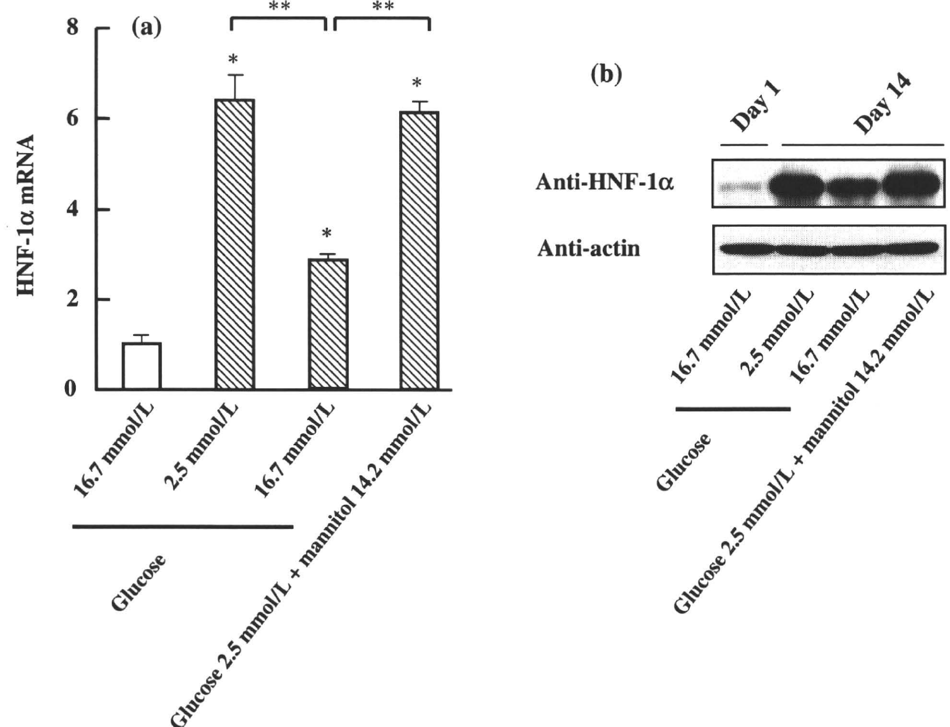

Fig. 2 Hepatocyte nuclear factor (HNF)-1α mRNA expression (a) and protein levels (b) in Caco-2 cells cultured in the presence of 2.5 or 16.7 mmol/L glucose on Day 1 (□) or 14 (▨) of confluence. Data are the mean±SEM from six independent experiments. *$P < 0.01$ compared with 16.7 mmol/L glucose on Day 1 of confluence. **$P < 0.01$. The mRNA expression of HNF-1α was normalized against that in cells cultured in the presence of 16.7 mmol/L glucose on Day 1 of confluence, which was defined as one unit.

HNF-1αR271G has a dominant negative effect on wild-type HNF-1α by a promoter assay using DPP-IV promoter luciferase construct in Caco-2 cells. Varying amounts of mutant HNF-1αR271G constructs (0–1.0 μg) were cotransfected with a constant amount of wild-type HNF-1α (0.25 μg) into Caco-2 cells. There were no significant differences in the transactivation of DPP-IV promoter, regardless of whether the HNF-1αR271G construct was transfected (Fig. 3a). The HNF-1αR271G mutant dose-dependently reduced transactivation of the DPP-IV promoter, showing a strong dominant-negative effect on wild-type HNF-1α (Fig. 3a). These findings indicate that HNF-1αR271G not only has lesser transactivation activity on the DPP-IV promoter, but also has a dominant-negative effect on wild-type HNF-1α.

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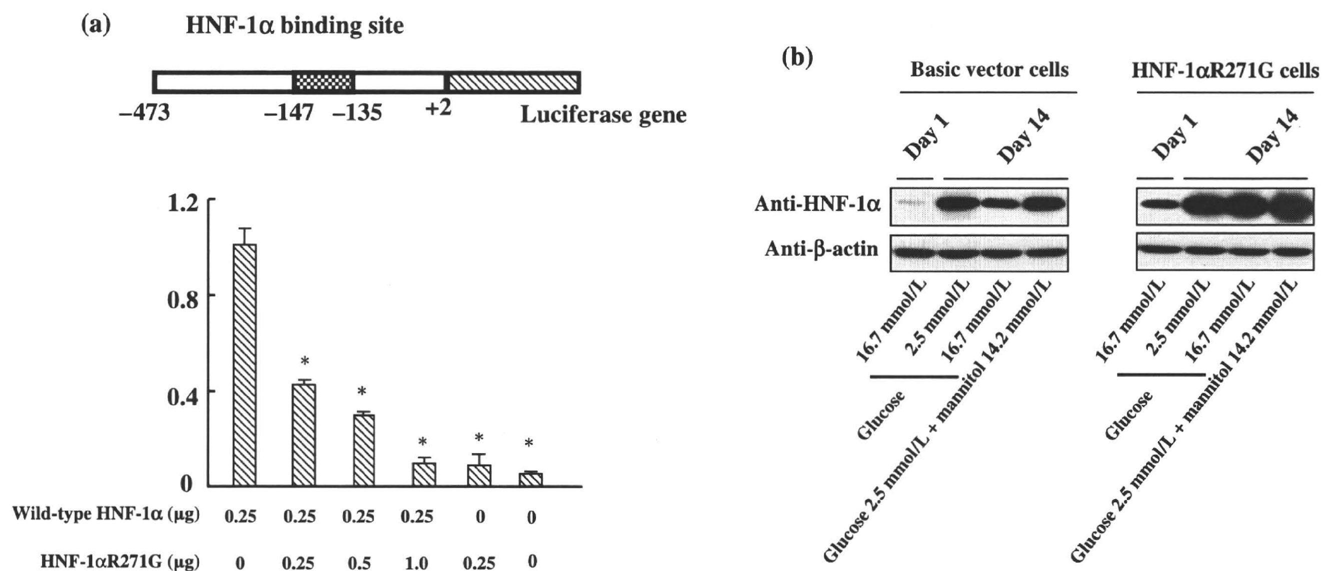

Fig. 3 Effects of mutant HNF-1αR271G on wild-type hepatocyte nuclear factor (HNF)-1α (a) and detection of mutant HNF-1αR271G gene expression in stably transfected cells by immunoblot (b). Wild-type HNF-1α (0.25 μg) and β-galactosidase construct (0.25 μg) were cotransfected with increasing amounts (0, 0.25, 0.5, 1.0 μg) of mutant HNF-1αR271G in Caco-2 cells. The total amount of plasmid was adjusted to 2.0 μg using pCMV6b vector. Data are the mean±SEM from six independent experiments. $*P < 0.01$ compared with wild-type HNF-1α (0.25 μg) alone.

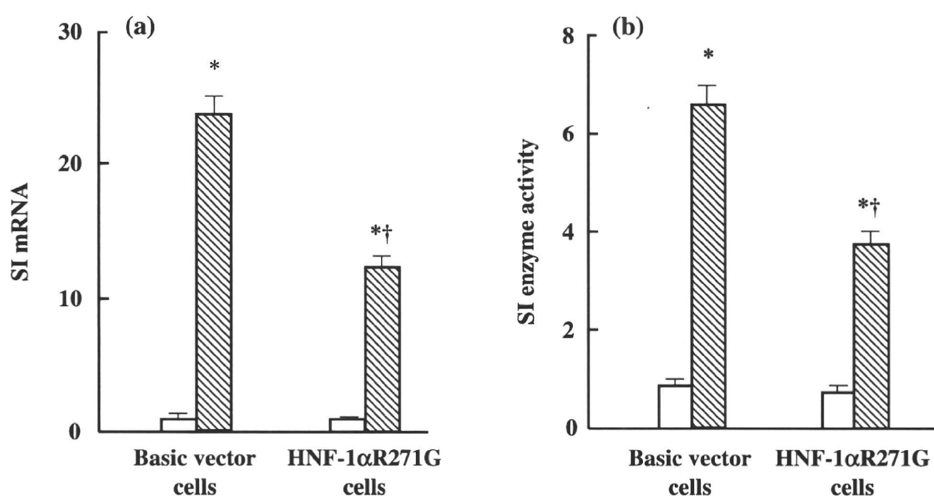

Fig. 4 Sucrase-isomaltase (SI) gene expression (a) and enzyme activity (b) in basic vector- and HNF-1αR271G-transfected cells cultured in the presence of 16.7 mmol/L glucose on Day 1 (□) or 14 (▧) of confluence. Sucrase-isomaltase gene expression and enzyme activity were normalized against that in basic vector-transfected cells on Day 1 of confluence, which was defined as one unit. Data are the mean±SEM from six independent experiments. $*P < 0.01$ compared with Day 1 of confluence; $^{\dagger}P < 0.01$ compared with basic vector cells on Day 14 of confluence.

Dominant-negative mutant HNF-1αR271G gene expression in stable cells

We planned to clarify whether glucose regulation of DPP-IV gene expression was affected when the function of HNF-1α was inhibited by dominant-negative mutant HNF-1αR271G. In preconfluent undifferentiated Caco-2 cells, endogenous DPP-IV gene expression and enzyme activity were not altered, despite transient overexpression of the mutant HNF-1αR271G gene (data not shown). To resolve this question, we produced two cell lines in which the dominant-negative mutant HNF-1αR271G and basic vector (as a control) were transfected. Stable constitutive expression of the mutant HNF-1αR271G gene was confirmed in protein level on Days 1 and 14 of confluence (Fig. 3b). Because the HNF-1α promoter site was used in the HNF-1αR271G expression vector, the expression pattern of the HNF-1αR271G gene in HNF-1αR271G cells was similar to that of HNF-1α in basic vector cells (Fig. 3b). With differentiation of HNF-1αR271G cells, an increased expression of the HNF-1αR271G gene was observed in protein level (Fig. 3b), and presumably inhibited endogenous HNF-1α function.

Confirmation of endogenous HNF-1α dysfunction in HNF-1αR271G cells

To confirm whether endogenous HNF-1α function is specifically inhibited by transfection of dominant-negative mutant HNF-1αR271G, we examined SI gene expression and enzyme activity in HNF-1αR271G cells. On Day 1 of confluence, there were no differences between basic vector and HNF-1αR271G cells in terms of SI gene expression or enzyme activity (Fig. 4). However, SI gene expression and enzyme activity were significantly lower in HNF-1αR271G cells than basic vector cells on Day 14 of confluence (Fig. 4). This indicates that endogenous HNF-1α function was specifically inhibited by transfection of the dominant-negative mutant HNF-1αR271G in HNF-1αR271G cells.

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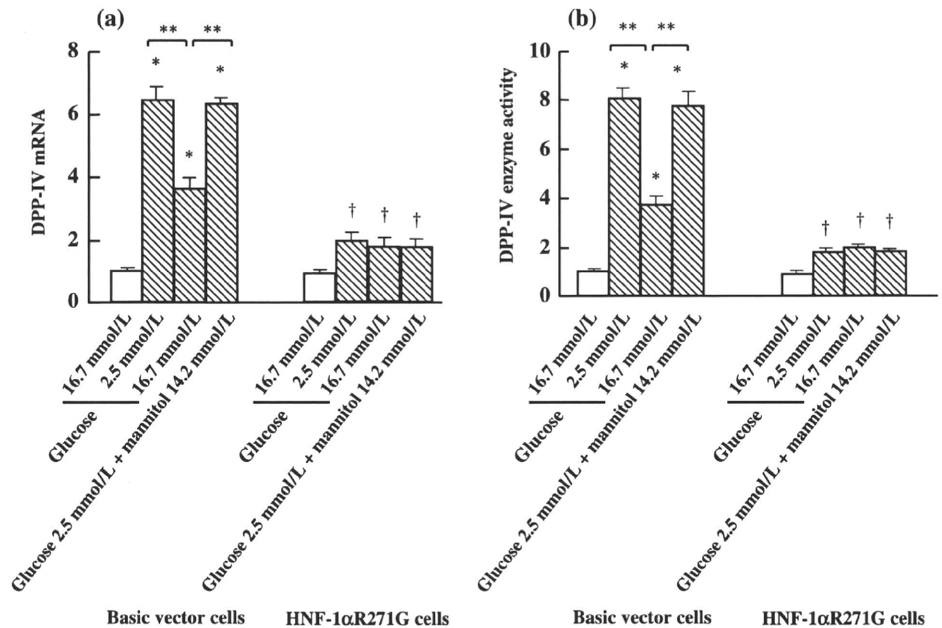

Fig. 5 Dipeptidyl peptidase IV (DPP-IV) gene expression (a) and enzyme activity (b) in basic vector- and HNF-1αR271G-transfected cells cultured in the presence of 2.5 and 16.7 mmol/L glucose on Day 1 (□) or 14 (▧) of confluence. Data are the mean±SEM from six independent experiments. †$P < 0.05$, *$P < 0.01$ compared with the corresponding cells cultured in the presence of 16.7 mmol/L glucose on Day 1 of confluence; **$P < 0.01$. Dipeptidyl peptidase IV gene expression and enzyme activity were against that of cells cultured in the presence of 16.7 mmol/L glucose on Day 1 of confluence, which was defined as one unit.

Effects of glucose on DPP-IV gene expression in HNF-1αR271G cells

To clarify changes in glucose regulation of DPP-IV gene expression in HNF-1αR271G cells, basic vector and HNF-1αR271G cells were cultured in the presence of 2.5 or 16.7 mmol/L glucose or 2.5 mmol/L glucose plus 14.2 mmol/L mannitol until Day 14 of confluence. On Day 1 of confluence, no differences were observed in DPP-IV gene expression and enzyme activity between basic vector and HNF-1αR271G cells (Fig. 5). On Day 14 of confluence, DPP-IV gene expression was markedly decreased, resulting in reduced DPP-IV enzyme activity in HNF-1αR271G cells compared with that in basic vector cells (Fig. 5). The glucose regulation of DPP-IV gene expression was confirmed in basic vector cells on Day 14 of confluence. However, it was compromised in HNF-1αR271G cells (Fig. 5). No differences were detected in DPP-IV gene expression and enzyme activity in HNF-1αR271G cells, regardless of glucose concentration (Fig. 5). This suggests that endogenous HNF-1α dysfunction compromises the glucose regulation of DPP-IV gene expression in Caco-2 cells.

DISCUSSION

Degradation of GLP-1 by DPP-IV causes a deficiency of early post-prandial insulin secretion and aggravates the diabetic condition. Previous clinical studies have reported that, among Type 2 diabetic patients, reduced DPP-IV activity has been found in subjects with poor glycaemic control and elderly obese subjects.[9,17] Although high glucose concentrations and hyperosmolarity induced by high glucose concentrations regulate the expression of a number of genes *in vivo* and *in vitro*,[18,19] it is not known whether high glucose concentrations and high glucose-induced hyperosmolarity regulate DPP-IV gene expression and enzyme activity. In the present study, we found that the transcription level of DPP-IV gene expression was lower in Caco-2 cells cultured in the presence of 16.7 mmol/L glucose compared with cells cultured in the presence of either 2.5 mmol/L glucose or 2.5 mmol/L glucose plus 14.2 mmol/L mannitol, resulting in reduced DPP-IV activity. This suggests that, in Caco-2 cells, high glucose concentrations, but not high glucose-induced hyperosmolarity, suppress DPP-IV gene expression, resulting in decreased DPP-IV enzyme activity. An electrophoretic mobility shift assay (EMSA) study has shown that HNF-1α has a binding site in the (–147/–135) fragment of the DPP-IV gene promoter.[20] In a previous study, we reported that HNF-1α is able to promote DPP-IV gene expression in Caco-2 cells.[10] In the present study, HNF-1α gene expression was also lower in Caco-2 cells cultured in the presence of 16.7 mmol/L glucose compared with 2.5 mmol/L glucose or 2.5 mmol/L glucose plus 14.2 mmol/L mannitol. The pattern of gene expression pattern of DPP-IV and its regulation by glucose is similar to that for HNF-1α in Caco-2 cells. Therefore, we hypothesize that glucose regulation of DPP-IV gene expression is mediated by HNF-1α.

To elucidate the hypothesis mentioned above that glucose regulation of DPP-IV gene expression is mediated by HNF-1α, we investigated whether glucose regulation of DPP-IV gene expression in Caco-2 cells was affected when the function of endogenous HNF-1α was inhibited. Specific inhibition of endogenous HNF-1α by transfection of a dominant-negative mutant HNF-1αR271G was confirmed by downregulation of SI gene expression and enzyme activity in HNF-1αR271G cells. Hepatocyte nuclear factor-1α directly binds to two sites (SIF2, SIF3) of the promoter of the SI gene and upregulates SI gene expression.[21] A reduction in SI gene expression and enzyme activity has been observed in Caco-2 cells in which a dominant-negative mutant of HNF-1α was stably expressed.[22]

Although HNF-1α promotes DPP-IV gene expression,[10] no significant differences were observed in DPP-IV gene expression and enzyme activity in stably transfected cells on Day 1 of confluence. At the beginning of differentation, little DPP-IV gene expression was found because expression of the DPP-IV gene in Caco-2 cells is closely associated with their differentiation state.[23] This may be one reason why expression of the HNF-1αR271G mutant did not affect DPP-IV gene expression and enzyme activity on Day 1 of confluence. However, on Day 14 of confluence, DPP-IV gene expression and enzyme activity in Caco-2 cells were lower in HNF-1αR271G-transfected cells compared with those transfected with basic vector

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and cultured in the presence of the same concentration of glucose. This indicates that transfected HNF-1αR271G had a dominant-negative effect on endogenous HNF-1α in the stable cells. The glucose regulation of DPP-IV gene expression was confirmed in basic vector-transfected cells. However, glucose regulation of DPP-IV gene expression was compromised in HNF-1αR271G-transfected cells. No differences were observed in DPP-IV gene expression and enzyme activity in HNF-1αR271G cells on Day 14 of confluence, regardless of the concentration of glucose in which the cells were cultured. Therefore, it is thought that inhibition of HNF-1α function by the dominant-negative mutant HNF-1αR271G is responsible for attenuation of glucose regulation of DPP-IV gene expression in HNF-1αR271G cells. These results support our hypothesis that glucose regulation of DPP-IV gene expression in Caco-2 cells is mediated by HNF-1α.

There is little information on DPP-IV enzyme activity in patients with Type 2 diabetes. Of patients with Type 2 diabetes, a reduction in DPP-IV enzyme activity has been observed in patients with poor glycaemic control, as well as in elderly obese patients;[9,17] however, another report has suggested that patients with Type 2 diabetes have increased DPP-IV enzyme activity.[6] The results of the present study do not rule out the possibility that DPP-IV enzyme activity may be increased in Type 2 diabetes, but hyperglycaemia seems not to be a causative factor for increased DPP-IV enzyme activity. In patients with Type 2 diabetes, glucose tolerance and insulin resistance may contribute to altered DPP-IV enzyme activity;[24,25] in addition, the effect of therapeutic agents on DPP-IV enzyme activity should not be overlooked.[26,27] Prevention of the inactivation of GLP-1 by direct inhibition of DPP-IV enzyme activity has emerged recently as a new treatment for Type 2 diabetes.[8] Although the results of the present study suggest that hyperglycaemia may reduce DPP-IV enzyme activity in patients with Type 2 diabetes, there is no doubt that DPP-IV inhibitors are useful therapeutic agents for Type 2 diabetes. Many clinical studies have reported that DPP-IV inhibitors significantly augment exogenously administered GLP-1, resulting in increased endogenous GLP-1 concentrations in the plasma and stimulating glucose-induced insulin secretion in Type 2 diabetes patients.[28,29] There are no inhibitors of DPP-IV gene expression available, because the molecular mechanism of DPP-IV gene expression has not yet been fully uncovered. The results of the present study indicate that HNF-1α is apparently an effective target in the development of DPP-IV inhibitors and its inhibition may lead to the suppression of DPP-IV gene expression.

In the present study, we found that glucose regulation of DPP-IV gene expression in Caco-2 cells is mediated by HNF-1α. This finding may help elucidate the molecular mechanism of glucose regulation of DPP-IV gene expression. The glucose-responsive element has not been identified in the HNF-1α gene promoter. In addition, the signalling pathways involved in glucose regulation of DPP-IV gene expression have not been fully elucidated and further detailed studies are required.

ACKNOWLEDGEMENTS

The authors thank Dr Jun Takeda (Gifu University, Gifu, Japan) for providing wild-type HNF-1α and mutant HNF-1αR271G cDNA clones. This study was supported by a post-doctoral fellowship for foreign researchers in Japan (to NG) from the Japan Society for the Promotion of Science (JSPS).

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