

PanK4 inhibits pancreatic β -cell apoptosis by decreasing the transcriptional level of pro-caspase-9

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Dear Editor:

Coenzyme A (CoA) is a primary and predominant acyl group carrier involved in a wide variety of important biochemical processes. The CoA biosynthetic pathway is composed of five enzymatic steps, of which Pantothenate kinase (PanK) is a key regulatory enzyme. The multiple isoforms of PanK are encoded by four different genes [1,2]. In our previous studies of SNP markers by genotyping the case-controlled DNAs, we found that one SNP within the hPANK4 gene on chromosome 1 was associated with type 2 diabetes [3-5]. We subsequently showed that rat PanK4 (rPanK4) was up-regulated when rats were challenged by high concentration of glucose [6]. M2-type pyruvate kinase (Pkm2) was found, both *in vitro* and *in vivo*, to be associated with rPanK4 [7]. These data suggest that PanK4 may have a role in diabetes pathogenesis. The development of type 2 diabetes is due partly to the loss of the pancreatic β -cell mass, therefore the secreted amount of insulin is insufficient to maintain the glucose homeostasis [8]. In the present study, we evaluated the effect of rPanK4 on β -cell apoptosis. We aimed to determine the potential of rPanK4 gene in β -cell apoptosis induced by the cytotoxic agent streptozotocin (STZ).

We generated a rat RINm5F cell line (insulin-secreting tumor cell line) overexpressing recombinant rPanK4 (rPanK4-RINm5F). Cells transfected with empty vector (empty-RINm5F) were used as the negative control (Fig-

ure 1A). We examined the effects of rPanK4 on cellular proliferation by MTT assay. Data shows that there were little differences in rates of cellular proliferation comparing the control and rPanK4-RINm5F cells. Accordingly, we focused on the potential role of rPanK4 in apoptosis process. Here, we used FACS to investigate the role of rPanK4 in STZ-induced apoptosis. RINm5F cells were exposed to a series of concentrations of STZ at the different time points. The apoptosis rate of rPanK4-RINm5F cells was significantly reduced compared to control cells only when cells were exposed to 2.5 mg/ml of STZ for 24 h. When RINm5F and empty-RINm5F transfected cells were exposed to 2.5 mg/ml of STZ, the apoptosis rate increased from $6.1 \pm 0.7\%$ to $19.5 \pm 0.9\%$ and from $7.3 \pm 0.4\%$ to $20.3 \pm 0.7\%$ respectively. By contrast, the apoptosis rate of the rPanK4-RINm5F cells only slightly increased to $11.1 \pm 1.2\%$ ($p < 0.01$) (Figure 1B1). The rPanK4 protective effect was further evaluated by using TUNEL and DNA ladder assays. The apoptosis (Figure 1B2, B4, B5, B6) and DNA fragmentation (Figure 1B3) were significantly decreased in rPanK4-overexpressing cells when exposed to 2.5 mg/ml of STZ for 24 h.

We further examined the effects of rPanK4 on apoptosis-related genes by quantitative real-time PCR. Overexpression of rPanK4 significantly reduced the expression level of the pro-caspase-9 mRNA by more than 50%, but not other apoptosis-related genes, including Bcl-2, Bax, Bad, pro-caspase-3, and pro-caspase-8 genes (Figure 1C). At protein level, pro-caspase-9 was significantly decreased in rPanK4-RINm5F cells. However, the pro-caspase-3 expression levels were not significantly different between rPanK4-RINm5F and RINm5F cells (Figure 1D1).

We analyzed the caspase activation by colorimetric and western blot assays. Caspase-3 and caspase-9 activities

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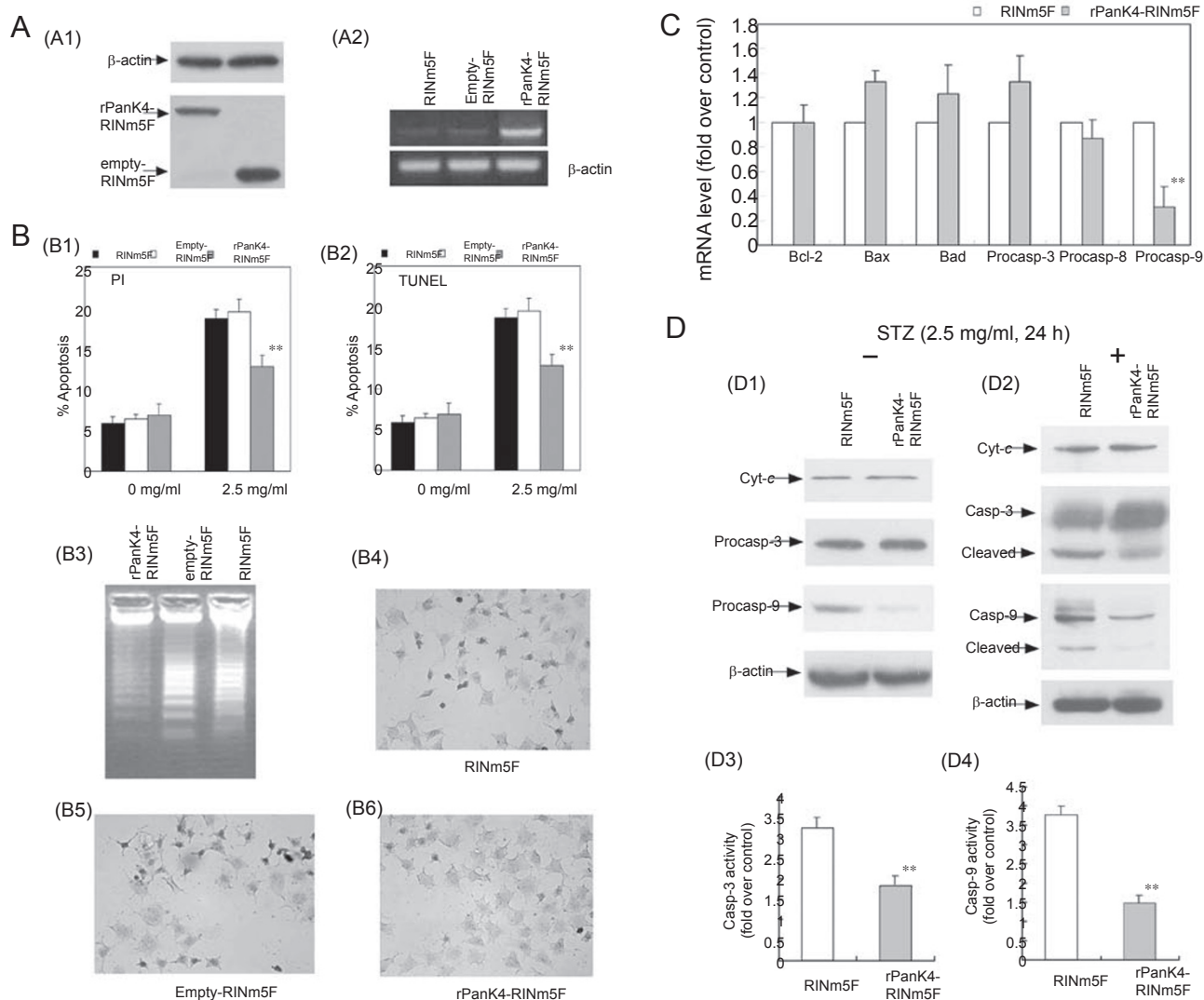


Figure 1 (A) Stable expression of rPanK4 in RINm5F cells. (A1) Western blot of EGFP protein (27 kDa) expressed in empty-vector transfected cells, and pEGFP-rPanK4 fusion protein (103 kDa) in cells transfected with the rPanK4-pEGFP vector. (A2) RT-PCR detection of rPanK4 in RINm5F cells. (B) Effects of rPanK4 on STZ-induced apoptosis. (B1) Apoptotic cells labeled with PI were quantified by FACS. (B2) The summary of apoptosis detected by the TUNEL assay. (B3) Detection of apoptotic cells by DNA ladder assay. (B4, 5, 6) Representative microscopic views of apoptosis after the TUNEL staining ($\times 400$). (C) Real-time PCR detection of apoptosis-related genes. (D) Western blotting of apoptosis-related proteins. (D1, 2) Western blotting of cytochrome *c*, caspase-9 and caspase-3. (D3) Detection of caspase-3 activity. (D4) Detection of caspase-9 activity. ** $p < 0.01$ compared with control by using unpaired Students' *t*-test.

were found decreased in rPanK4-RINm5F cells compared to RINm5F cells stimulated with STZ (Figure 1D2, D3, D4). Taken together, it might imply that overexpression of rPanK4 partially inhibited the caspase-3 and caspase-9 activities in STZ-induced apoptosis in RINm5F cells. Moreover, the decrease in the caspase-3 activity was correlated with the decreased caspase-9 activity.

To test whether the decrease of caspase-9 activity was due to the changes of cytochrome *c*, we compared the

level of cytochrome *c* in cytosol in RINm5F and rPanK4-RINm5F cells by western blot. Figure 1D shows that the level of cytochrome *c* in rPanK4-RINm5F cells was similar to that in the control cells. The result of cytosol cytochrome *c* also suggested that overexpressing rPanK4 had little effect on the mitochondrial function.

In our previous studies, PanK4 was shown to be related to diabetes. Interestingly, PanK4 does not possess a kinase activity and thus does not change cell proliferation/apop-

tosis via its activity in CoA metabolism [9]. Evidence with the *Drosophila* orthologue of PanK gene *fumble* (*fb1*) suggested a CoA-independent role in cell proliferation [10]. We showed that STZ-stimulated apoptosis was significantly blocked by an overexpression of rPanK4. In the present study, rPanK4 expression led to decreased pro-caspase-9 mRNA level. Accordingly, the expression of pro-caspase-9 protein also decreased. Consistently, rPanK4-overexpressing cells underwent significantly lower caspase-3 activation when treated with STZ.

In conclusion, our current study suggests that rPanK4 has an anti-apoptotic effect, via down-regulating caspase-9. Given this novel finding, future studies are needed to elucidate the biological functions and molecular mechanisms of PanK4 in type 2 diabetes.

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