Fanconi anemia complementation group C protection against oxidative stress-induced β-cell apoptosis

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Abstract. Diabetes mellitus (DM) and other glucose metabolism abnormalities are commonly observed in individuals with Fanconi anemia (FA). FA causes an impaired response to DNA damage due to genetic defects in a cluster of genes encoded proteins involved in DNA repair. However, the mechanism by which FA is associated with DM has not been clearly elucidated. Fanconi anemia complementation group C (FANCC) is a component of FA nuclear clusters. Evidence suggests that cytoplasmic FANCC has a role in protection against oxidative stress-induced apoptosis. As oxidative stress-mediated β-cell dysfunction is one of the contributors to DM pathogenesis, the present study aimed to investigate the role of FANCC in pancreatic β-cell response to oxidative stress. Small interfering RNA-mediated *FANCC* suppression caused a loss of protection against oxidative stress-induced apoptosis, and that overexpression of *FANCC* reduced this effect in the human 1.1B4 β-cell line. These findings were confirmed by Annexin V-FITC/PI staining, caspase 3/7 activity assay, and expression levels of anti-apoptotic and pro-apoptotic genes. *Insulin* and *glucokinase* mRNA expression were also decreased in FANCC-depleted 1.1B4 cells. The present study demonstrated the role of FANCC in protection against oxidative stress-induced β-cell apoptosis and established another mechanism that associates FANCC deficiency with β-cell dysfunction. The finding that FANCC overexpression reduced β-cell apoptosis advances the potential for an alternative approach to the treatment of DM caused by FANCC defects.

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Introduction

Fanconi anemia (FA) is a genetic disorder that is caused by an abnormality in genes encoding Fanconi anemia proteins (FA proteins), which comprise a multi-protein nuclear complex that has an essential role in DNA inter-strand crosslink repair (1,2). Dysfunction of FA proteins leads to cytogenetic instability, chromosomal breakage, defective DNA repair and cellular hypersensitivity to DNA crosslinking agents (3), and these effects are known to cause developmental defects, bone marrow failure, aplastic anemia and increased cancer risk in individuals with FA (4-6). To a lesser extent, metabolic disorders associated with FA, including diabetes mellitus (DM), hyperglycemia and insulin disorders, have been reported in ~40% of patients with FA (7-9).

FA complementation group C (FANCC) is a component of the FA multiprotein nuclear complex (2). However, it has been demonstrated that instead of FANCC being an isoform targeted to the nucleus, FANCC localization in cytoplasm is essential for the correction of enhanced cytotoxicity of crosslinks in cells with predominant defects in the *FANCC* gene (10). Additionally, cytoplasmic FANCC has a role in prevention of oxidative DNA damage (11), and protection against oxidative stress-induced apoptosis (12). Reactive oxygen species (ROS) accumulation and cell apoptosis have been identified in many cell types with FANCC depletion. These cell types include hematopoietic progenitor cells, hepatocytes, and murine embryonic fibroblasts isolated from FA model mice (13-16). It was suggested that FANCC counteraction of oxidative stress is mediated through glutathione S-transferase P1 (GSTP1) and nicotinamide adenine dinucleotide phosphate-cytochrome P450 reductase (17,18). FANCC significantly increased the catalytic activity of GSTP1, which is an inducible antioxidant enzyme that has a major role in the intracellular defense of toxin and ROS (19). Fancc$^{-/-}$ cells were hypersensitive to oxidant stimuli and underwent enhanced oxidant-mediated apoptosis compared with the wild type controls as a result of oxidative stress activating the redox-dependent apoptosis signal-regulating kinase 1 pathway (15). Wang *et al* (20) reported that overexpression of *FANCC* protected hematopoietic progenitors from death induced by Fas-mediated apoptosis. A previous study demonstrated that FANCC

associated with uncoordinated-5A protein, a pro-apoptotic dependent receptor, and delayed apoptosis in neuroblastoma SH-SY5Y cell line (21). These findings indicate that oxidative stress is a contributor to FA pathogenesis, and that FANCC is a key contributor to oxidative stress response mechanisms in various cell types.

Oxidative stress has also been implicated as an important factor in the progression of DM via interference with insulin signal transduction, insulin production and induction of β-cell apoptosis (22,23). Oxidative stress and DM are commonly observed in patients with FA, and high levels of ROS were detected in insulin-sensitive tissues of Fancc$^{-/-}$ mice (24). Furthermore, insulin receptor and insulin receptor substrate 1 tyrosine phosphorylation were impaired in *Fancc* knockout mice treated with tumor necrosis factor (TNF)-α compared with their wild-type littermates (24). It was concluded that defects in *Fancc* led to ROS accumulation in insulin target cells, thus interfering with the insulin signaling pathway, leading to insulin resistance (24).

While most studies in the literature focused on the actions of FANCC on signal-transduction in insulin-responsive cells, none has explored the role of FANCC in β-cells with limited expression of antioxidant enzymes [reviewed by Robertson and Harmon (25)]. In the present study, it was hypothesized that depletion of FANCC causes pancreatic β-cell hypersensitivity to oxidative stress-induced apoptosis. Accordingly, the aim of the present study was to investigate the role of FANCC in pancreatic β-cell response to oxidative stress.

Materials and methods

Study approval. The present study was conducted at the Faculty of Medicine Siriraj Hospital, Mahidol University (Bangkok, Thailand). Siriraj Hospital is Thailand's largest university-based national tertiary referral center. The protocol for the present study was approved by the Siriraj Institutional Review Board (COA no. Si491/2014).

Cell culture. Human 1.1B4 pancreatic β-cell line (American Type Culture Collection, Manassas, VA, USA) was maintained in RPMI-1640 medium (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) containing 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.), 100 IU/ml penicillin, and 100 mg/ml streptomycin at 37˚C in a 95% humidified atmosphere containing 5% CO_2.

Small interfering RNA (siRNA) transfection. Human FANCC SMARTpool ON-TARGETplus siRNA (siRNA-FANCC; cat. no. L-011033-00-0005) and siRNA ON-TARGETplus Non-targeting Pool (siRNA-control; cat. no. D-001810-10-20) were purchased from Dharmacon (GE Healthcare; Dharmacon, Inc., Lafayette, CO, USA). The day prior to transfection, $1.6x10^5$ cells/well were seeded into 6-well plates. Knockdown was performed at 24 and 48 h after seeding. siRNA-FANCC and siRNA-control were transfected at a concentration of 100 pmol using Lipofectamine® 2000 Transfection Reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. At 48 h after transfection, the cells were harvested and FANCC expression levels were determined by western blot analysis.

Plasmid construct and transfection. To generate FANCC recombinant plasmids, FANCC coding sequence NM_000136.2 and FLAG-tag sequence DYKDDDDK were amplified with the following primer sequences: Forward, 5'-CGGGATCCATGGCTCAAGATTCAGTAG-3' and reverse, 5'-GCTCTAGACTACTTATCGTCGTCATCCTTG TAATCGACTTGAGTTCGCAGCTCTTTAAGG-3'. The product was cloned into pcDNA3.1 plasmids (Invitrogen; Thermo Fisher Scientific, Inc.). All plasmids were amplified in *Escherichia coli* (DH5α) and purified using QIAamp DNA Mini Kit (Qiagen GmbH, Hilden, Germany). The day prior to transfection, $2x10^5$ cells/well were seeded into 6-well plates. After 24 h, the cells were transfected with 500 ng FANCC-constructed plasmid or pcDNA3.1 empty plasmid using the same conditions as the ones described for siRNA transfection. At 48 h after transfection, the cells were harvested and FANCC protein expression levels were determined by western blot analysis.

RNA isolation and RT-qPCR. Total RNA from 1.1B4 cells ($5x10^5$) was isolated using TRIzol Reagent (Invitrogen; Thermo Fisher Scientific, Inc.). Subsequently, RNA was reverse transcribed into cDNA using a RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Inc.) Gene-specific primer pairs were designed using Primer3Plus program (www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) or derived from previous studies (26-28). All primers (Table I) were checked against National Center for Biotechnology Information Primer-BLAST. RT-qPCR was performed in a LightCycler 480 Instrument (Roche Diagnostics GmbH, Mannheim, Germany) using LightCycler® 480 SYBR Green I Master (Roche Diagnostics GmbH). Initial enzyme activation proceeded at 95˚C for 10 min, followed by 45 cycles at 95˚C for 30 sec, 60-62˚C for 20 sec, and 72˚C for 20 sec. β-actin was used as an internal control to normalize input cDNA. Relative mRNA expression levels were calculated using the $2^{-\Delta\Delta Cq}$ method (29). All assays were performed in triplicate.

Western blot analysis. Total protein was extracted by lysing cells in radioimmunoprecipitation assay lysis buffer (Thermo Fisher Scientific Inc.). Protein concentrations were quantified by Bradford protein assay (Bio-Rad Laboratories, Inc., Hercules, CA, USA) using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Inc.). Proteins (50-100 μg) were separated onto 10% SDS polyacrylamide gel, and then electroblotted onto nitrocellulose membrane (Bio-Rad Laboratories, Inc.) using a semi-dry electrophoretic transfer cell (Bio-Rad Laboratories, Inc.). Primary goat polyclonal antibody against human FANCC (C-14; cat. no. sc-18110; 1:500; Santa Cruz Biotechnology Inc., Dallas, TX, USA), mouse monoclonal antibody against FLAG-tag (1:2,000; cat. no. F3165; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany), and mouse monoclonal antibody against β-actin (1:1,000; cat. no. s-47778; Santa Cruz Biotechnology, Inc.) were incubated with the membranes for 2 h at room temperature. Following this, membranes were incubated with horseradish peroxidase-linked rabbit anti-goat or goat anti-mouse secondary antibody (1:1,000; cat. no. P044701-2; Dako; Agilent Technologies, Inc., Santa Clara, CA, USA) for 1 h at room temperature. Binding antibodies were visualized using an enhanced chemiluminescence detection kit (SuperSignal

Table I. Primers and conditions for reverse transcription-quantitative polymerase chain reaction.

First author, year	Gene	Primer (5'-3')	Annealing temperature (˚C)	Product size (bp)	(Refs.)
Present study	*FANCC* (NM_000136)	F: TCATCGCTGCCTCAAGC R: GGAACCAGCTCTAAAGGG	62	352	-
Robertson, 2007	*GCK* (NM_033508)	F: TGGACCAAGGGCTTCAAGGCC R: CATGTAGCAGGCATTGCAGCC	60	207	(25)
Robertson, 2007	*INS* (NM_000207)	F: TACCAGCATCTGCTCCCTCT R: TGCTGGTTCAAGGGCTTTAT	60	120	(25)
Vasu, 2013	*BCL-2* (NM_000657)	F: TTTGAGTTCGGTGGGGTCAT R: TGACTTCACTTGTGGCCCAG	62	275	(26)
Vasu, 2013	*BAX* (NM_138763)	F: TGGCAGCTGACATGTTTTCTGAC R: TCACCCAACCACCCTGGTCTT	62	195	(26)
Floros, 2006	*DKK1* (NM_012242)	F: TCACGCTATGTGCTGCCCCG R: TGAGGCACAGTCTGATGACCGGA	62	223	(27)
Present study	*β-actin* (NM_001101)	F: AGAAAATCTGGCACCACACC R: CTCCTTAATGTCACGCACGA	62	395	-

FANCC, Fanconi anemia complementation group C; F, forward; R, reverse; GCK, glucokinase; INS, insulin; BCL2, BCL2 apoptosis regulator; BAX, BCL2 associated X, apoptosis regulator; DKK1, dickkopf WNT signaling pathway inhibitor 1.

West Pico PLUS Chemiluminescent Substrate; Roche Diagnostics GmbH), and bands were detected by biomolecular imager (ImageQuant LAS 4010; GE Healthcare Life Sciences, Little Chalfont, UK). β-actin staining was used as an internal control in all experiments. Expressed bands were quantified by computer-assisted scanning densitometry using ImageJ version 1.4.3.x (National Institutes of Health, Bethesda, MD, USA).

Flow cytometry. Following knockdown or overexpression of *FANCC* in 1.1B4 β-cells for 48 h, $1.4x10^6$ cells were treated with 0.5 mM H_2O_2 for 4 h. Subsequently, Annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI) staining was performed using Annexin V-FITC/PI Apoptosis Detection Kit (ImmunoTools GmbH, Friesoythe, Germany) according to the manufacturer's protocol. Apoptosis was analyzed using a BD FACSVerse flow cytometer (BD Biosciences, San Jose, CA, USA) and FlowJo software version 10.4 (FlowJo LLC, Ashland, OR, USA). Each experiment was independently performed three times.

Caspase activity. Luminescent type Caspase-Glo 3/7 Assay (Promega Corporation, Madison, WI, USA) was used to measure caspase 3/7 activity. Cells were seeded in a Corning 96-well white flat bottom plate (Corning Life Sciences, Tewksbury, MA, USA) at $8x10^3$ and $1x10^4$ cells per well for knockdown and overexpression, respectively. Following 24 h, the cells were transfected with 500 ng plasmids (FANCC-pcDNA3.1 or empty vector) or 100 pmol siRNA (siRNA-FANCC or siRNA-control). In order to increase the population of cells successfully transfected with either FANCC-pcDNA3.1, empty vector, siRNA-FANCC or siRNA-control, the transfected cells were transfected twice with the same type and amount of plasmid 24 h after initial transfection. At 24 h after the second transfection, cells were treated with 0.5 mM H_2O_2 for 4 h. Following this, 100 μl Caspase-Glo 3/7 Reagent (Promega Corporation) was added into each well. The plate was then shaken at 300 to 500 rpm in a dark room for 30 min at room temperature (~25˚C). Luminescence was measured using a multi-detection microplate reader (Synergy H1; BioTek Instruments, Inc., Winooski, VT, USA).

Statistical analysis. Data analysis was performed using SPSS Statistics version 18.0 (SPSS, Inc., Chicago, IL, USA). Student's t-test was used to evaluate differences of the mean ± standard error of the mean. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Increased apoptosis of FANCC-deficient β-cells. In order to investigate the role of *FANCC* in β-cell response to oxidative stress, FANCC-depleted 1.1b4 cells were generated via siRNA-mediated transient silencing. Knockdown efficiency is demonstrated in Fig. 1A. The results demonstrated that *FANCC* knockdown cells exhibited increased apoptosis compared with si-control cells, in non-induced and H_2O_2-induced oxidative stress conditions (Fig. 1B-D). In non-induced condition, Annexin V-FITC/PI staining revealed 12.6 and 1.6% of control cells to be in late and early apoptotic states, respectively; while, 13.6 and 5.3% of FANCC knockdown cells were in late and early apoptotic states, respectively (Fig. 1B, left). Under H_2O_2-induced oxidative stress induction, 19.9 and 2.7% of control cells were in late and early apoptotic states, respectively, while 24.9 and 6.7% of FANCC knockdown cells were in late and early apoptotic states, respectively (Fig. 1B, right). Overall, in non-induced condition, ~14.2 and ~18.9% of control cells and *FANCC* knockdown cells, respectively, were apoptotic ($P<0.05$). Under oxidative stress induction, the percentage of apoptotic cells in control cells and *FANCC*

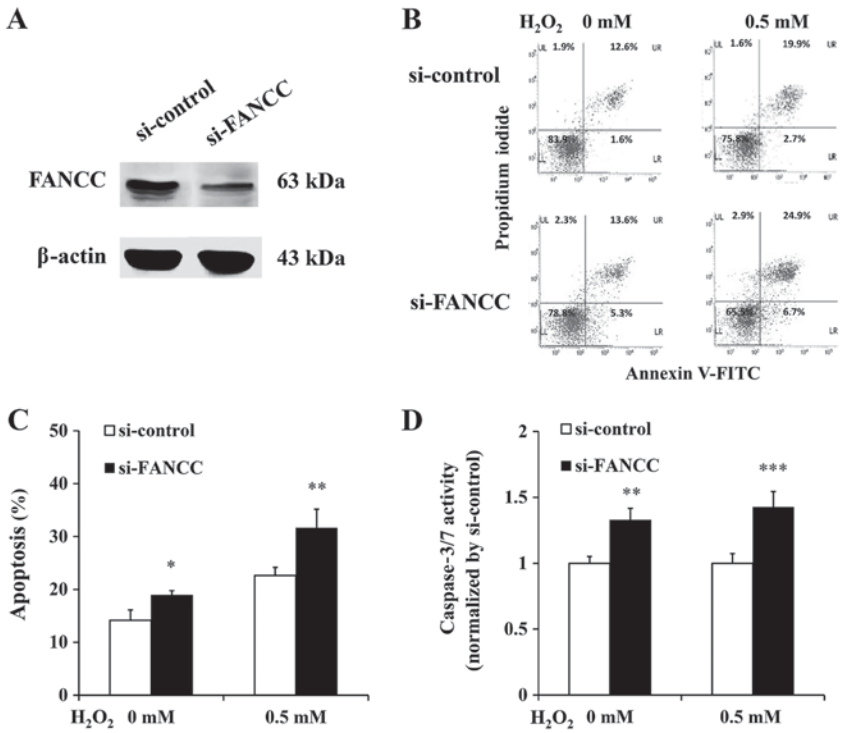

Figure 1. Knockdown of *FANCC* increases β-cell apoptosis. (A) Representative western blot images demonstrating the successful knockdown of *FANCC* in 1.1B4 β-cells. (B) Annexin V-FITC/propidium iodide staining was used to analyze apoptotic cells by flow cytometry and (C) quantify the percentage of apoptotic cells. (D) Caspase 3/7 activity in *FANCC* knockdown cells normalized to the corresponding si-control group. Data are presented as the mean ± standard error. *P<0.05, **P<0.01, ***P<0.001 vs. si-control. FANCC, Fanconi anemia complementation group C; si, small interfering RNA; FITC, fluorescein isothiocyanate.

knockdown cells increased to 22.6 and 31.3%, respectively (P<0.01; Fig. 1C). Caspase 3/7 activities of *FANCC* knockdown cells were 1.33-fold and 1.43-fold higher than control cells under non-induced and H_2O_2-induced oxidative stress conditions, respectively (P<0.01 and P<0.001, respectively; Fig. 1D).

Overexpression of FANCC protects β-cells from oxidative stress-induced apoptosis. To further elucidate the effect of FANCC in attenuating β-cell apoptosis, plasmid construct for *FANCC* overexpression was transfected into 1.1B4 β-cells, and the percentage of apoptosis in cells overexpressing *FANCC* was investigated. Annexin V-FITC/PI staining (Fig. 2A and B) demonstrated that 1.1B4 β-cells overexpressing *FANCC* had lower total percentage of apoptotic cells than control cells transfected with empty vector (22.5 vs. 25.1%, respectively; P=0.067). Additionally, under oxidative stress induction, the percentage of apoptotic cells in 1.1B4 β-cells overexpressing *FANCC* was significantly lower than that of control cells transfected with empty vector (25.7 vs. 32.2%, respectively; P<0.05; Fig. 2C). In addition, 1.1B4 β-cells overexpressing *FANCC* had lower caspase 3/7 activity compared to control cells (0.75-fold; P<0.05) under H_2O_2-induced oxidative stress conditions (Fig. 2D). However, the significant protective effect of FANCC against apoptosis was not observed in non-induced condition.

Depletion of FANCC alters expression of genes involved in insulin expression, secretion and apoptosis pathways. To confirm the anti-apoptotic effect of FANCC in the human β-cell line, the expression of anti-apoptotic [*BCL2 apoptosis regulator* (*BCL-2*)] and pro-apoptotic genes [*BCL2 associated X, apoptosis regulator* (*BAX*)] were investigated. The results demonstrated that in FANCC-deficient 1.1b4 β-cells, *BCL-2* mRNA expression decreased by 2.59 fold (P<0.001), while *BAX* mRNA level increased by 1.93 fold (P<0.01), compared to the expressions of control cells (Fig. 3). Depletion of FANCC also led to a 3.15-fold increase in *dickkopf WNT signaling pathway inhibitor 1* (*DKK1*) expression (P<0.01). As *DKK1* encodes a negative regulator of Wnt signaling, a pathway implicated in β-cell apoptotic defenders (30), increased *DKK1* expression level in FANCC-deficient cells thereby reaffirms the crucial role of FANCC in protection against β-cell apoptosis. Notably, the expression levels of *insulin (INS)* and *glucokinase* (*GCK)* genes, which are critical for insulin synthesis and secretion, were decreased [0.77-fold (P<0.01) and 0.82-fold (P<0.05), respectively] compared to cells transfected with siRNA-control.

Discussion

DM and abnormalities of glucose and insulin metabolism are common among patients with FA (7-9). However, little is known regarding the role of FA proteins in maintenance of glucose homeostasis. While previous studies focused on the link between ROS accumulation and insulin resistance in FANCC-deficient state (24), the results of the current study provide evidence linking FANCC insufficiency to DM via deterioration of β-cell ability to defend against oxidative stress-induced apoptosis.

Pancreatic β-cells have inherently low levels of anti-oxidants (31), which may be due to the need to maintain a low level of ROS to facilitate insulin production. Accordingly,

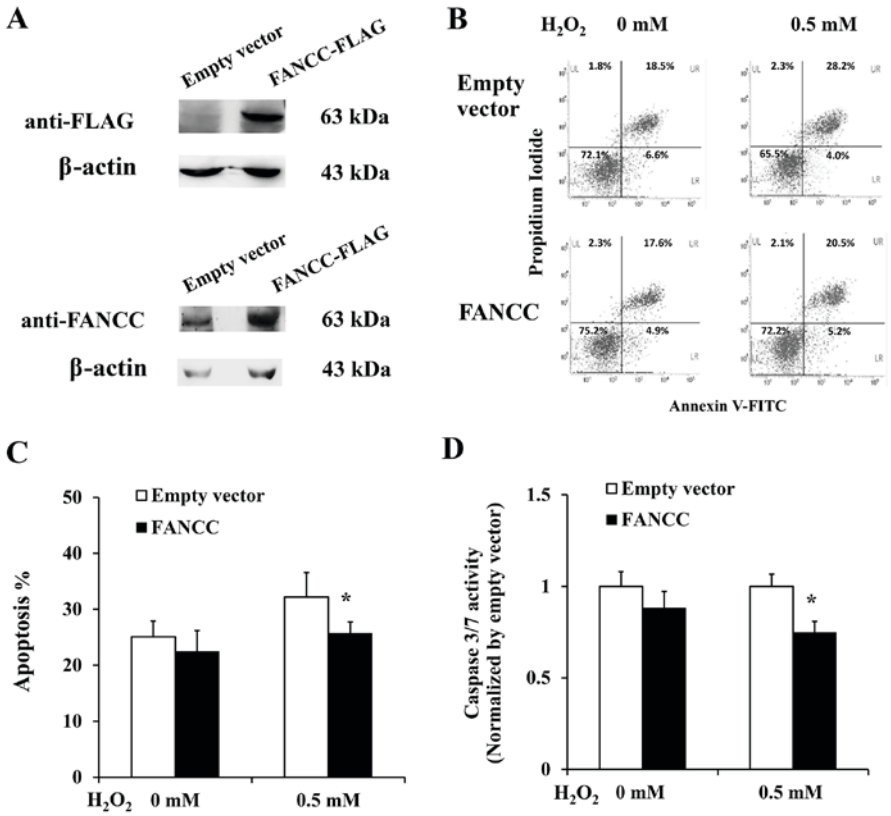

Figure 2. Overexpression of *FANCC* protects 1.1b4 β-cells from apoptosis following treatment with H_2O_2. (A) Representative western blot images illustrating the successful transfection with FLAG-tagged FANCC plasmid. (B) Annexin V-FITC/PI staining was used to analyze apoptotic cells by flow cytometry and (C) quantify the percentage of apoptotic cells. (D) Caspase 3/7 activity following transfection with *FANCC* plasmid normalized to the corresponding empty vector group. Data are presented as the mean ± standard error. *P<0.05 vs. empty vector. FANCC, Fanconi anemia complementation group C; si, small interfering RNA; FITC, fluorescein isothiocyanate.

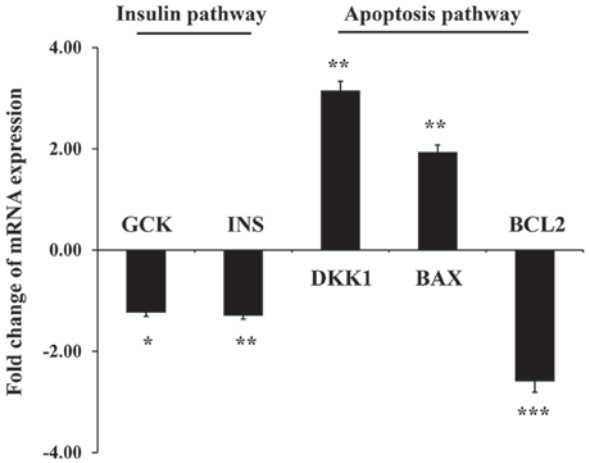

Figure 3. Up and downregulation of genes involved in insulin (*GCK, INS*) and apoptosis pathways (*DKK1, BAX, BCL2*) investigated in *FANCC*-depleted 1.1B4 β-cells, as measured by reverse transcription-quantitative polymerase chain reaction experiments. Data are presented as mRNA expression fold change (mean ± standard error) in *FANCC*-depleted cells relative to cells expressing siRNA-control. *P<0.05, **P<0.01, ***P<0.001 vs. si-RNA control. FANCC, Fanconi anemia complementation group C; siRNA, small interfering RNA; GCK, glucokinase; INS, insulin; DKK1, dickkopf WNT signaling pathway inhibitor 1; BAX, BCL2 associated X, apoptosis regulator; BCL2, BCL2 apoptosis regulator.

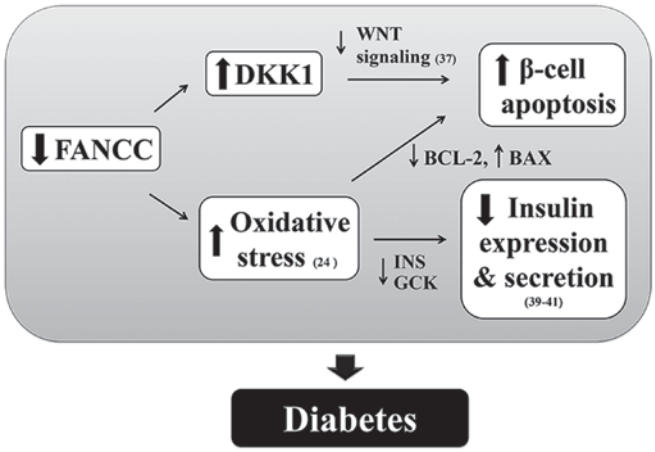

Figure 4. Schematic diagram illustrating the mechanistic link between FANCC-depleted cells and diabetes mellitus in 1.1B4 human pancreatic β-cells. FANCC, Fanconi anemia complementation group C; DKK1, dickkopf WNT signaling pathway inhibitor 1; INS, insulin; GCK, glucokinase; BCL2, BCL2 apoptosis regulator; BAX, BCL2 associated X, apoptosis regulator.

this specialized cell type is extremely susceptible to oxidative stress. Previous studies addressed the deleterious effects of oxidative stress on β-cell function and survival (24,32), and a link between inability of β-cells to adequately secrete insulin and the development of type 2 DM was demonstrated (33,34). Furthermore, hypersensitivity to oxidative agents and enhancement of oxidant-mediated apoptosis were

evident in FANCC-deficient cells isolated from mice (35). In addition, Zhang *et al* (36) demonstrated that TNF-α-induced senescence was associated with the accumulation of ROS and oxidative DNA damage in *Fancc*$^{-/-}$ mice compared with wild-type littermates. In the present study, the role of FANCC in antioxidant defense mechanisms of the human 1.1b4 β-cell line was investigated. It was also demonstrated that without H_2O_2-induced oxidative stress, siRNA-mediated *FANCC* suppression led to a significant increase in β-cell apoptosis, while transient overexpression of *FANCC* decreased β-cell apoptosis. However, the significant protective effect of FANCC overexpression and empty vector against apoptosis was not observed in non-induced conditions. This may be due to the presence of endogenous FANCC expression in 1.1b4 cells, which may have been sufficient enough to prevent apoptosis in physiological conditions. These results suggest the necessity of a physiological level of FANCC as a requirement for β-cell survival. Under oxidative stress-induced conditions, apoptosis was more pronounced in FANCC-depleted cells, while FANCC-overexpressed cells were resistant to H_2O_2-induced oxidative stress. These data reaffirm the critical role of FANCC in protection against oxidative stress-induced β-cell apoptosis. However, the data indicated a minor increase of apoptosis level in FANCC knockdown cells as compared with control cells. Potentially, other pathways besides FANCC are involved in cell apoptosis or there may be compensatory mechanisms that counteract the apoptosis. Additionally, the increased or decrease in caspase 3/7 activities were marginal, although statistically significant. BCL2 expression was decreased and BAX expression was increased >2-fold in FANCC knockdown cells compared with controls. The difference in apoptotic indicator expression is not surprising, as BCL2 and BAX were detected at the mRNA level while caspase 3/7 activity was determined at the protein level; furthermore, numerous molecules and signaling pathways are involved. However, it can be concluded that lack of FANCC increases caspase 3/7 activity, increases BAX and decreases BCL-2 RNA level.

Although a comprehensive and conclusive mechanism that explains how FANCC facilitates β-cell survival was not elucidated in the present study, the finding that *DKK1* expression level was increased in FANCC-depleted β-cells suggests the possibility of Wnt signal transduction involvement, which required further investigation. *DKK1* is known to be a Wnt signaling antagonist that sequesters Wnt receptors (30,37). Upregulation of *DKK1* inhibits Wnt signal transduction, thus promoting apoptosis (38), and *DKK1* upregulation was observed in FANCC-depleted cells (39). FANCC forms a complex with C-terminal-binding protein-1 and β-catenin, and acts as a transcriptional repressor that directly inhibits *DKK1* expression (40). It is, therefore, possible that FANCC modulates *DKK1* expression via nuclear activity. The exact mechanism by which FANCC regulates *DKK1* requires further investigation.

In addition to loss of protection against oxidative stress-induced apoptosis in FANCC-depleted 1.1B4 β-cells, the expression of genes involved in insulin synthesis (such as, *INS*), a key hormone for regulating glucose uptake into peripheral cells, and secretion (such as, *GCK*), a rate-limiting enzyme in glycolysis that is a sensor of glucose-stimulated insulin secretion (41), was decreased. Reduction in *INS* and *GCK* expression is common in pancreatic β-cells exposed to oxidative stress, and in islets isolated from DM subjects (42-44). Taken together, the data from the present study reveal an association between FANCC depletion and loss of oxidative defense in pancreatic β-cells and reduction in insulin production (Fig. 4). Furthermore, the mechanism underpinning the association of *FANCC* knockdown with upregulation of DKK1 in human β-cells will be explored in future studies. These findings may explain the development of DM and abnormal glucose metabolism in patients with FA (7). Finally, the finding that FANCC overexpression reduced β-cell apoptosis advances the possibility of an alternative approach to the treatment of DM caused by FANCC defects.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

SK, PTY and NP conceived and designed the study. SK performed the cell experiments and molecular biology analysis. PJ and WT analyzed and interpreted the data. SK was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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