Gemcitabine Resistance Induced by Interaction between Alternatively Spliced Segment of Tenascin-C and Annexin A2 in Pancreatic Cancer Cells

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Received January 7, 2010; accepted May 25, 2010

Pancreatic cancer is the fourth leading cause of cancer-related death in the western countries and it is resistant to almost all cytotoxic drugs. In the current study, we explored the gemcitabine resistance induced by the interaction between Annexin A2 (ANXA2) and alternatively spliced segment of tenasin-C (TNfnA-D). In the pancreatic cancer cell culture system in vitro, it was proved that exogenous recombinant TNfnA-D combined with the cell surface ANXA2 specifically and their interaction suppressed gemcitabine-induced cytotoxicity on pancreatic cancer cells in a dose-dependent manner. Meanwhile, the TNfnA-D/ANXA2 interaction increased the phosphorylation of phosphatidylinositol 3-kinase (PI3K), Akt, inhibitory xB (1xB) kinase αβ (IKKαβ), 1xBα, and p65 nuclear factor-xB (NF-xB) significantly. Inhibition of Akt and PI3K with their specific inhibitors partially reversed the suppression of gemcitabine-induced cytotoxicity elicited by TNfnA-D/ANXA2 interaction. Activation of p65 NF-xB was dependent on the phosphorylation of PI3K/Akt. The phosphorylated IKKαβ induced the phosphorylation and degradation of 1xBα, the sequential phosphorylation, nuclear translocation and activation of p65 NF-xB. Pyrrolidine dithiocarbamate (PDTC) effectively blocked the activity of p65 NF-xB in response to TNfnA-D. Down-regulation of p65 NF-xB with its specific small interfering RNA (siRNA) restored the gemcitabine-induced cytotoxicity suppressed by TNfnA-D/ANXA2 interaction. For the first time, this study shows that ANXA2/TNfnA-D interaction induced gemcitabine resistance via the canonical PI3K/Akt/NF-xB signaling pathways in pancreatic cancer cells. Therefore, therapy targeting ANXA2/TNfnA-D and/or p65 NF-xB may have potential clinical application for patients with pancreatic cancers.

Key words gemcitabine; pancreatic cancer; Annexin A2; tenasin-C; drug resistance

Although pancreatic cancer accounts for only 2% of all malignancies, it is one of the most aggressive and lethal cancers known today, with a 5-year survival of less than 5%. It is the fourth most common cause of cancer-related death in the western countries. Patients with pancreatic cancer typically have poor prognosis partly because the cancer usually causes no symptoms early on and it is already metastatic at the time of diagnosis. The treatment options for pancreatic cancer include surgery, radiation and chemotherapy.1 Generally, gemcitabine was chosen as the first-line therapeutic drug to treat pancreatic cancer. However, the one-year survival of pancreatic cancer patients treated with gemcitabine was only about 18%. One of the major reasons for the poor therapeutic effects on pancreatic cancer was its high resistance to gemcitabine.2

Dozens of studies had been focused on the mechanisms of intrinsic or acquired gemcitabine resistance in pancreatic cancer. Among them, Annexin A2 (ANXA2), a member of the annexin family, was once identified to be involved in anti-apoptotic effects on pancreatic cancer cells through its ligand progastrin.3 The annexin family members were calcium-dependent phospholipid-binding proteins. They played important roles in the regulation of cell growth and signal transduction pathways. ANXA2 was involved in diverse cellular processes such as cell motility, linkage of membrane-associated protein complexes to the actin cytoskeleton, endocytosis, fibrillobinolysis, ion channel formation and cell matrix interactions.4 ANXA2 had been proposed to function inside the cell in sorting of endosomes and outside the cell in anticoagulant reactions.5,6 Additionally, enhanced expression of ANXA2 in human pancreatic carcinoma cells and primary pancreatic cancers was observed.7 ANXA2 overexpression predicted an rapid recurrence after surgery in pancreatic cancer patients undergoing gemcitabine-adjutnant chemotherapy.8

Ligands for ANXA2 were varied. The alternatively spliced segment of tenasin-C (TNfnA-D) was proved to be a receptor for ANXA2 with high affinity.9 Tenasin-C (TN-C) was an important component of the provisional extracellular matrix (ECM) that characterized solid tumors. TN-C was a hexameric glycoprotein with a molecular weight of 210—400 kDa and was reported transiently present during ECM remodeling, embryo maturation, inflammation and neoplasias.10 Each subunit of TN-C contained a TA domain that formed a coiled-coil at the N-terminus, 14/15 epidermal growth factor (EGF)-like domains, 8—15 fibronectin type III-like (FNIII) repeats (depending on alternative RNA splicing) and a fibrinogen-like domain (FBG). The universal FNIII domains (FNIII repeats 1—5 and FNIII repeats 6—8) were present in all TN-C variants. The largest TN-C variant also contains nine alternatively spliced domains (FNIII repeats A1-D, TNfnA-D, Fig. 1A) which were missing in the shortest splice variant. A small-molecular-weight variant of TN-C without TNfnA-D domains existed constitutively in normal tissues, whereas the large-molecular-weight variants, including different combinations of alternative spliced FNIII repeats, were specifically expressed in tissues on certain pathological conditions and called large TN-C variants.11 Large TN-C transcripts were found in pancreatic cancer and in chronic pancreatitis, but not in the normal pancreas.12 A recent study proved deposited fibrillar TN-C could promote pancreatic cancer progression.13

In colon cancer, ANXA2 overexpression was found corre-
lated with stromal TN-C overexpression and taken as a worse prognostic marker. Similarly, the expression of TN-C and cell surface annexin II were increased in the progression from low-grade PanIN lesions to pancreatic cancer. When considering the chemoresistance induced by the interaction between progastrin and ANXA2, we wondered whether TNfnA-D/ANXA2 interaction would elicit similar phenomenon. In this study, sera concentrations of large TN-C variants of patients with pancreatic cancers were evaluated. Meanwhile, the human pancreatic cancer lines were cultured in vitro and the recombinant TNfnA-D was added. Finally, we reported for the first time ANXA2/TNfnA-D interaction induced gemcitabine resistance via the canonical phosphatidylinositol 3-kinase (PI3K)/Akt/nuclear factor-κB (NF-κB) signaling pathways in pancreatic cancer cells.

**MATERIALS AND METHODS**

**Patients and Normal Subjects** The sera were collected from 60 patients resistant to gemcitabine therapy (25 female and 35 male; median age, 55.3 years; range, 52–65 years), 32 patients sensitive to gemcitabine therapy (11 female and 21 male; median age, 56.6 years; range, 48–67 years) with pancreatic cancer and 30 healthy volunteers (20 female and 10 male; median age, 50.2 years; range, 42–58 years) served as controls and kept in −70 °C for storage. All patients were informed consent according to hospital guidelines.

**Sandwich Enzyme-Linked Immunosorbent Assay (ELISA)** Levels of Large TN-C variants were assessed with the sandwich ELISA kit (IBL, Japan) according to the manufacturer’s instructions. And the final results were read at a 450 nm wavelength.

**Cell Culture and Treatment** Human pancreatic cancer lines were all purchased from ATCC (Rockville, MD, U.S.A.). AsPC-1, Capan-2, PANC-1, MIA PaCa-2, BxPC-3 were grown in RPMI 1640 (Invitrogen, Carlsbad, CA, U.S.A.) supplemented with 10% (v/v) heat-inactivated fetal calf serum (Hyclone, Logan, UT, U.S.A.). All cells were maintained at 37 °C in a humidified atmosphere with 5% CO₂. Cell viability was routinely checked after passage by trypan blue exclusion and was consistently >95%. For signaling pathways analysis, cells were pre-treated with PI-3K inhibitor LY294002 (20 μM), AKT inhibitor (20 μM) or NF-κB inhibitor Pyrrolidine dithiocarbamate (PDTC, 25 μM) (all purchased from Calbiochem, La Jolla, CA, U.S.A.) for 60 min, and then exposed to 20 μg/ml recombinant TNfnA-D. Following culturing for variable time periods, the cells were harvested.

**Cell Proliferation Assays** Cell proliferation was quantified by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Sigma Chemical Co., St. Louis, MO, U.S.A.). Logarithmically growing cells were plated into 96-well plates at 10³ cells/well and allowed to adhere for 6 h. Cells were cultured in medium containing 10% fetal bovine serum (FBS) and 0–10 μM gemcitabine. The medium was replaced every 48 h and cellular proliferation was determined after 96 h. After treatment, 10 μl of the MTT was added to each well to assess the cell viability, and after 4 h at 37 °C, the purple–blue MTT formazan precipitate was dissolved in 100 μl of dimethyl sulfoxide (DMSO), and the optical density was measured with a V₅₀ microplated spectrophotome-
ter (Molecular Devices, Sunnyvale, CA, U.S.A.) at 570 nm, referenced to 650 nm. Each experiment was repeated at least three times. The 50% inhibitory concentration of gemcitabine (IC_{50}) was calculated using Microsoft Excel software for semi-log curve fitting with regression analysis.

**Flow Cytometric Analysis** Cells were incubated with anti-Annexin A2 (H-50, Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.) or its corresponding control antibodies (Abs) at 4 °C for 30 min, respectively. After washing twice, the cells were suspended in Dulbecco phosphate-buffered saline (DPBS) containing a working dilution of fluorescein isothiocyanate (FITC)-labeled goat anti-rabbit immunoglobulin G (IgG) antibodies (Merek Research Laboratories, Rayway, NJ, U.S.A.) and incubated for 30 min at 4 °C. Cells were washed, fixed and then analyzed on a FACScan cytometer (Coulter Electronics, Inc., Hialeah, FL, U.S.A.). The apoptosis of cells was analyzed by an Annexin V/propidium iodide (PI) staining method.

**Proteins and Antibodies** The cDNA of TNfnA-D was gifted by Dr. Xin Xie from the Northwest University of China and inserted into a pSecTag2B plasmid (Invitrogen). The corresponding recombinant TNfnA-D protein (shown in Fig. 1) was expressed in COS-7, a cell line established from monkey kidney cells of the African green monkey, as a 6×His-tagged fusion protein and purified with the His GraviTrap affinity column (GE Healthcare Bio-Sciences Co., Piscataway, NJ, U.S.A.) according to the manufacturer's protocol. The purified TNfnA-D protein were identified by a monoclonal antibody (mAb) specific for TNfnD (gifted by Dr. YuCai Wang[5]) with Western blot. Annexin II was purified from bovine lung as described previously.[9] Anti-TNfnA-D and anti-ANXA2 sera were routinely prepared in rabbits. Affinity-purified anti-TNfnA-D or anti-ANXA2 antibodies were prepared on a column of TNfnA-D or ANXA2-coupled CNBr-activated Sepharose. The bound antibodies were eluted with 0.1 M glycine (pH 2.6) and neutralized.

**Western Blot Analysis** Antibodies for western blot included polyclonal anti-inhibitory kBα (1xBα) (C-21), anti-p50 NF-κB (H-119), anti-phosphorylated Akt1/2/3 (Thr308)-R, anti-Akt1/2 (H-136), anti-PI3K p85α (Z-8) antibodies from Santa Cruz Biotechnology (Santa Cruz, CA, U.S.A.) and monoclonal anti-phosphorylated p65 NF-κB (Ser536, 7F1), anti-phosphorylated 1xBα (Ser32/36, 5A5), polyclonal anti-phosphotyrosine p85 PI3K, anti-IKKα/β, and anti-phosphorylated IKKα/β (Ser176/180) antibodies from Cell Signaling Technology (Beverly, MA, U.S.A.). The harvested cells were lysed with NE-PER™ or MEM-PER™ protein extraction reagent (Pierce Chemical, Rockford, IL, U.S.A.), supplemented with protease inhibitor cocktail (Sigma), and their protein concentrations were determined by BCA assay (Pierce). The protein lysates were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto nitrocellulose membranes. After blocking, the target proteins were probed with 1:1000 antibodies mentioned above overnight at 4 °C, and then incubated with horseradish peroxidase (HRP)-conjugated antimouse or rabbit antibodies at room temperature for 1 h. The bound antibodies were visualized by using LumiGlo reagent (Pierce) and the relative levels of each protein to the β-actin were analyzed.

**Binding of Activated p65 NF-κB to DNA Assay** Activation of p65 NF-κB was examined using TransAM p65 NF-κB Chemi Transcription Factor Assay ELISA kit from Active Motif (Carlsbad, CA, U.S.A.) as per the manufacturer's instructions. Briefly, 30 μl of complete binding buffer were added into each well coated with immobilized oligonucleotide containing the NF-κB consensus site (5'-GGGACTTTCC-3'). Nuclear proteins (20 μl), prepared from cells incubated with or without recombinant TNfnA-D for different time periods, were added to the wells in 80 μl complete lysis buffer and incubated for 1 h at room temperature. The wells were washed and incubated with anti-p65 NF-κB antibody (1:1000 dilution) for 1 h and washed three times again followed by incubation with HRP-conjugated secondary antibody (1:1000; 50 μl) for 1 h. The wells were washed four times followed by the addition of the chemiluminescent working solution. The resulting chemiluminescence, proportional to the levels of nuclear p65 NF-κB, bound to the consensus sequence, was measured with a luminometer.

**Promoter-Reporter Assay for Verifying the Transcriptional Activity of p65 NF-κB** Transcriptional activity of p65 NF-κB was determined with RapidReporter pRR-High-NFκB (Active Motif) according to the manufacturer's instructions. Briefly, the RapidReporter plasmid was transfected into the plated cells. Twenty-four hours after transfection, the cells were treated with or without recombinant TNfnA-D for 4 h (optimal for measuring maximum activity of nuclear NF-κB as determined from previous experiments). Cells were lysed to measure luciferase levels with a luminometer.

**Down-Regulation of p65 NF-κB by Small Interfering RNA** The p65 NF-κB small interfering RNA (siRNA; GC-CCUAUCCCUUACGGAUCA, from Thermo Scientific Dharmacon, Lafayette, CO, U.S.A.) was used to examine the role of p65 NF-κB in mediating the antiapoptotic effects. Cells were seeded in 35-mm dishes and incubated at 37 °C for 24 h followed by growth in serum- and antibiotic-free medium for 24 h. The cells were then transfected with either 100 nmol/l p65 NF-κB siRNA construct or a negative control siRNA construct (Dharmacon) using 4 μl DharmaFECT transfection reagent 4 (Dharmacon). After 48 h, the siRNA-transfected cells were treated with or without recombinant TNfnA-D for 48 h. The cells were treated with gemcitabine for 4 h to induce apoptosis. Total cellular proteins were extracted from gemcitabine-treated cells, and the relative levels of p65 NF-κB were measured by Western blot analysis as described above.

**Statistics** To compare values among multiple groups, one-way analysis of variance (ANOVA) was applied. For comparison of mean values between two groups, the unpaired t test was used. All values are mean ± S.E.M., except where otherwise indicated. Statistical significance was accepted at p<0.05.
present when compared the concentrations of large TN-C variants within patients resistant or sensitive to gemcitabine. Moreover, concentrations of large TN-C variants within patients resistant or sensitive to gemcitabine were both significantly higher than the normal controls (Table 1).

**TNfnA-D Combined with ANXA2 Specifically and Induced Gemcitabine-Resistance in a Dose-Dependent Manner** The membrane ANXA2 expression was quantified on the human pancreatic cancer lines AsPC-1, Capan-2, PANC-1, MIA PaCa-2 and BxPC-3 by flow cytometry. Owing to the highest positive percentage of ANXA2 expression on MIA PaCa-2 cells (about 42.3%, Fig. 1B), we chose MIA PaCa-2 to determine the effects of TNfnA-D/ANXA2 interaction on the gemcitabine 50% inhibitory concentration (IC50). Additionally, we also took another cell line Capan-2 as the cell models to repeat the following experiment and found similar roles played by canonical PI3K/Akt/NF-κB signaling pathways in inducing gemcitabine resistance (data not shown).

The MIA PaCa-2 cells were pre-treated with recombinant TNfnA-D (1—100 μg/ml) 24 h before gemcitabine addition and a dose-dependent effect of TNfnA-D on gemcitabine IC50 was observed. A significantly increased gemcitabine IC50 was present at the TNfnA-D concentration of 10 μg/ml (62.5±3.7 vs. 40.2±2.2 nm, p<0.05) and it reached a platform at 20 μg/ml (Fig. 1C).

To test whether TNfnA-D/ANXA2 interaction was responsible for the gemcitabine-resistance, 20 μg/ml rhTNfnA-D was preincubated with its antibodies or an equal amount of nonimmune IgG for 1 h before adding to the cells. Similarly, cells plated in the wells were preincubated with either anti-ANXA2 or control antibodies for 1 to 2 h followed by the addition of 20 μg/ml rhTNfnA-D for 24 h. The gemcitabine IC50 alteration was blocked by either anti-ANXA2 or anti-TNfnA-D antibodies rather than the control antibodies (Fig. 1D).

Table 1. Large TN-C Variants Concentrations in Subjects

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Normal controls</th>
<th>Resistant patients</th>
<th>Sensitive patients</th>
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<tbody>
<tr>
<td>TN-C (ng/ml)</td>
<td>30.2±8.9</td>
<td>132.7±17.4**</td>
<td>82.6±13.5**</td>
</tr>
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++ p<0.01 vs. the normal controls; ∗p<0.01 vs. the sensitive patients.

For signaling pathways analysis, cells were pre-treated with signaling transducer inhibitors for 60 min before exposure to recombinant TNfnA-D. Following culturing for variable time periods, the cells were harvested and analyzed. (A) TNfnA-D induced phosphorylation of PI3K and Akt; (B) PI3K and Akt inhibition reduced gemcitabine-resistance induced by TNfnA-D/ANXA2 interaction. ∗p<0.05; **p<0.01 vs. the control group.

A significant increase in the phosphorylated levels of IKKaβ was observed after 6 to 36 h after stimulation, with a peak at 6 h (Fig. 3A). The relative levels of phosphorylated IxBα were also significantly increased by approximately 2- to 3-fold at 6 h after TNfnA-D stimulation, and these levels remained elevated until 36 h after stimulation (Fig. 3B). At the same time, increased phosphorylation of IxBα was accompanied with a significant decline in the relative levels of total IxBα by 6 h after TNfnA-D stimulation, and the levels of total IxBα remained significantly low until 48 h after TNfnA-D stimulation (Fig. 3B).

In association with the degradation of IxBα, a significant increase in phosphorylation of cellular p65 NF-κB was measured in response to TNfnA-D, which also peaked at 6 h after TNfnA-D stimulation (Fig. 4A). A significant increase in relative levels of phosphorylated NF-κB (Fig. 4B) was measured in the nuclear fraction of cells after 6 h of stimulation with TNfnA-D. The increase in nuclear NF-κB remained elevated until 48 h after TNfnA-D treatment, suggesting a sustained phosphorylation of p65 NF-κB in response to TNfnA-D.
NF-κB Was Required for Gemcitabine-Resistance Induced by TNfnA-D/ANXA2 Interaction in Pancreatic Cancer Cells

An in vitro DNA-binding assay was conducted to confirm the binding capacity of translocated NF-κB. A significant increase in the binding of nuclear protein (NF-κB) was measured at 6 h after TNfnA-D stimulation, which was about 5-fold higher than that in control samples (Fig. 5A). Activation of NF-κB in terms of DNA binding remained elevated until 36 h after TNfnA-D treatment. In transient transfection assays with a promoter-reporter, a similar significant increase in the expression of luciferase was measured from 6 to 36 h (data not shown). When Akt or PI3K were inhibited with their inhibitors, activation of NF-κB in response to TNfnA-D was reversed by about 60% in a promoter-reporter transient transfection study (Fig. 5B).

NF-κB siRNA was applied for down-regulating expression of NF-κB to evaluate its function on mediating the gemcitabine resistance. MIA PaCa-2 cells transfected with NF-κB siRNA showed a loss of about 80% of NF-κB protein (Fig. 5C). Loss of NF-κB activity or expression induced by PDTC or its siRNA resulted in almost complete loss of suppression on gemcitabine-induced cytotoxicity elicited by TNfnA-D (Fig. 5D). These results thus confirmed for the first time that NF-κB was required for mediating protective (survival) effects of TNfnA-D on pancreatic cancer cells.

DISCUSSION

TNfnA-D expression was elevated in stroma of pancreatic cancer and ANXA2 played key roles in gemcitabine resistance. However, no direct relationship exited between TNfnA-D/ANXA2 interaction and gemcitabine resistance. When we assessed sera large TN-C variants collected from the patients undergoing gemcitabine therapy, we found significant difference was present between those resistant or sensitive to gemcitabine. These clinical data supplied us new clues for chasing exact mechanism behind these phenomena.

The Annexins are a family of Ca\(^2+\)/lipid binding proteins which differ from most of other Ca\(^2+\)-binding proteins in the Ca\(^2+\) binding cites. In the vertebrates, 12 annexin members (A1—A11, A13) have been identified. Annexin A2 had also been found outside the cellular environment besides inside the cells. Although increased ANXA2 expressions were...
found in the pancreatic cancer cells, they were indeed taken as the total ANXA2 expressions with Western blot. Here, for the first time, we proved a panel of pancreatic cancer cells all expressed extracellular membrane ANXA2 with moderate density directly by flow cytometry.

During pathogenesis of pancreatic cancers, ANXA2 played important roles through distinct ligands including antiapoptosis (when interacting with progastrin),16) cell motility (when interacting with S100A6),16) proliferation, invasion and angiogenesis (when interacting with tissue plasminogen activator, tPA).17—19) When alternatively spliced segment of tenascin-C (TNfnA-D) was first identified as the high affinity receptor for ANXA2, it was concluded that annexin II receptors on endothelial cells interacted with tenascin-C that mediated several cell regulatory functions such as mitogenesis, cell migration and loss of focal adhesions.20) In this study, we deduced that TNfnA-D/ANXA2 interaction induced another important consequence, i.e. chemoresistance. The membrane ANXA2 expression was not elevated by TNfnA-D addition (data not shown). Therefore, TNfnA-D increased gemcitabine IC50 and reached the platform at concentration of 20 μg/ml (Fig. 1C). We prepared the specific polyclonal antibodies against TNfnA-D or ANXA2 to block their activities, respectively. Interestingly, although no significant difference of gemcitabine IC50 was found between the groups treated with antibodies against TNfnA-D or ANXA2 (Fig. 1D), there was still light tendency of more effectiveness when anti-TNfnA-D antibodies were applied. We owed the phenomenon to potential distribution of other ligands for TNfnA-D on the pancreatic cells.

Several signaling pathways including Akt/PI3K, NF-κB, JAK/STAT, p38 mitogen-activated protein kinase (MAPK), and MEKK4 were activated when ANXA2 bound with its various ligands. At the same time, the NF-κB and Akt/PI3K signal transduction pathways were implicated in the resistance of numerous solid tumors against a variety of anticancer drugs. Previous study proved high basal NF-κB activities were exhibited in the gemcitabine-resistant pancreatic cancer cells.21) No related studies were focused on the sequential signaling pathways after TNfnA-D/ANXA2 combination. Here, we tried to explore whether NF-κB and Akt/PI3K signal transduction pathways were involved in the gemcitabine resistance.

PI3K/Akt acted as upstream of NF-κB. In a previous report, LY294002, a PI3K inhibitor, was found to have no effect on gemcitabine resistance in pancreatic cancer cells.22) However, in another3) and our studies, LY294002 decreased gemcitabine resistance induced by TNfnA-D/ANXA2. These discrepancies may be due to noncoordinate expression of IKKs, which was postulated to play a role in determining the cell type-specific role of PI3K/Akt in NF-κB activation. Cells with high proportion of IKKα (activated by Akt) to IKKβ were more sensitive to PI3K inhibitors and vice versa.23) Meanwhile, Akt inhibitor also reduced gemcitabine resistance. When compared with the almost complete abrogation of gemcitabine resistance induced by NF-κB inhibitor PDTC or its specific siRNA, neither LY294002 nor Akt inhibitor could not block gemcitabine resistance so efficiently. It should be due to that more upstream molecules took part in the TNfnA-D/ANXA2 signaling, which needed further investigation.

The IKK complex is composed of two kinase subunits (IKKα and IKKβ) and a regulatory subunit, IKKγ. In the classic NF-κB signaling pathway, IKKβ is both sufficient
and necessary for phosphorylating IkBα and IkBβ. Binding of IkB to NF-κB prevents NF-κB-IκB complex from translocating to the nucleus, thereby maintaining NF-κB in an inactive state. In MIA PaCa-2 cells, TNfα-D stimulated phosphorylation of IKKα/β, resulting in phosphorylation and degradation of IkBα followed by phosphorylation, nuclear translocation and activation of p65 NF-κB. Taken together, for the first time, our finding verified that TNfα-D/ANXA2 interaction induced gemcitabine resistance via the canonical PI3K/Akt/NF-κB signaling pathways in pancreatic cancer cells. The modulation of NF-κB by pharmacological or genetic approaches may have therapeutical potential when combined with gemcitabine in the treatment of pancreatic cancers.

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