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TNK1 Deficiency Inhibited Cell Survival, Clonogenic Assay and Effects Expression Levels of EMT Markers

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Abstract: *PRC is a part of the male reproductive system that helps make and store seminal fluid. In adult men, a typical prostate is about 3 centimetres long and weighs about 20 grams. It is located in the pelvis, below the urinary bladder and in front of the rectum. PRC is considered a malignant tumour because it is a mass of cells that can invade other parts of the body. PRC most commonly metastasizes to the bones, lymph nodes, and may into the rectum, bladder and lower ureters after local progression. The route of metastasis to the bone is thought to be venous as the prostatic venous plexus drairs into the prostate connecting with the vertebral veins. PRC is a zinc-accumulating, citrate-producing organ.*

The main function of non-receptor protein-tyrosine kinase (nRTKs) is to participate signal transduction in activated T-cells and B-cells in the immune system. Most of the nRTKs are localised in the cytoplasm. TNK1/Kos1 is a 72-KDa NRPTK located on the human chromosome 17p13.1. TNK1/Kos1 is a member of the Ack family, and it is involved in apoptosis and cell growth, but the results are controversial. Nuclear factor-κB and Ras may mediate these effects of TNK1.

To investigate the role of TNK1 in PC-3 cells, TNK1 deficient cells were used in the study. The results showed that TNK1 deficiency inhibited cell survival and clonogenicity of PC-3 cells; however, decreased expression EMT markers beta-catenin and vimentin in TNK1 deficient cells. These results suggest that TNK1 might contribute to the maligancy of tumor cells, therefore, TNK1 might be a potential prognosis factor for metastatic prostate cancer.

Keywords: *PRC / TNK1 / EMT / MTT / PC-3 cells.*

I. Introduction

1.1 Prostate cancer (PRC)

Prostate cancer (PRC) is the second most frequent tumour in males in the industrialized world [1-2]. An increase in this disease has been detected over the past few decades, probably in part due to a greater use of prostate-specific antigen (PSA) testing without ruling out the influence of unknown factors [3]. This higher rate of incidence is associated with an increase in the number of patients with the localized disease (gland and surrounding anatomical area) at diagnosis, permitting greater disease control by surgery and radiotherapy (RT). The classification in degrees of risk for localized disease and prognostic algorithms enables local treatment options to be offered with or without hormone therapy, which is currently the standard treatment [4].

PRC, the cells of these prostate glands mutate into cancer cells. PRC is classified as an adenocarcinoma, or glandular cancer, that begins when normal semen-secreting prostate gland cells mutate into cancer cells. The peripheral zone is the region of the prostate gland where the adenocarcinoma is most common. Initially, small clumps of cancer cells remain confined to otherwise normal prostate glands, a condition known as carcinoma in situ orprostatic intraepithelial neoplasia (PIN). The protein ZIP1 (Synaptonemal complex protein) is responsible for the active transport of zinc in prostate cells. One of zinc's important roles is to change the metabolism of the cell in order to produce citrate, an important component of semen.

The process of zinc accumulation, alteration of metabolism, and citrate production is energy inefficient, and prostate cells sacrifice enormous amounts of energy (ATP) in order to accomplish this task. Prostate cancer cells are generally devoid of zinc. The inability to male eitrate, PRC cells are able to save energy, and utilize the new abundance of energy to grow and spread. The absence of zinc is thought to occur via a silencing of the gene that produces the transporter protein ZIP1. ZIP1 is now called a tumour suppressor gene product for the gene SLC39A1(Solute carrier family 39 member 1). The cause of the epigenetic silencing is unknown. Strategies which transport zinc in transformed prostate cells effectively eliminate these cells in animals. Zinc inhibits NF-κB pathways (nuclear factor kappa-light-chain-enhancer of activated B cells), is anti-proliferative, and induces apoptosis in abnormal cells. Unfortunately, oral ingestion of zinc is ineffective since high concentrations of zinc in prostate cells is not possible without the active transporter ZIP1 [6]. Loss of cancer suppressor genes, early in the prostatic carcinogenesis, has been localized to chromosomes 8p, 10q, 13q, and 16q. P53 (Phosphoprotein p53) mutations in the primary prostate cancer are relatively low and are more frequently seen in metastatic settings, hence, p53 mutations are the latest events in the pathology of the PRC.

1.2 Tyrosine kinases

Tyrosine kinase is an enzyme able to convert a phosphate group from ATP to protein in the cell. It functions as an "on" or "off" switch in a lot of cellular functions. Tyrosine kinase is a subclass of protein kinase. The phosphate set is attached to the amino acid (tyrosine) on the protein. Tyrosine kinases are a subset of the larger category of protein kinase that attach phosphate sets to other amino acids (serine and threonine). Phosphorylation of proteins by kinases are a substantial mechanism in communicating signals within a cell (signal transduction) and regulating cellular activity, such as cell division. Protein kinases may mutate, become adhesive are in the "on" position, and cause unregulated cell growth, which is a necessary move for the evolution of cancer.

Classification, occurs pursued as active targets in cancer therapy due to the stridentdependence of cancer cells on one or more these structures for proliferation and survival. At least 90 unique tyrosine kinases are encoded by the human genome, of which 58 include the receptor-tyrosine kinases (RTKs)-grouped into 20 subfamilies, and 32 non-receptor tyrosine kinases (NRTKs) -grouped into 10 subfamilies based on the structure of the kinase domain [7-8].

Receptor tyrosine kinases (RTKs) are the upper-affinity cell surface receptors for a lot of the polypeptide growth factors, cytokines, and hormones. Of the 90 unique tyrosine kinase genes identified in the human genome, 58 encode receptor tyrosine kinase proteins [7]. RTKs organs, for instance cell's growth, proliferation, differentiation, adhesion, migration and apoptosis are critical components in the organization of the immune system. Tnk1 is a 72-KDa non–receptor protein tyrosine kinase (NRPTK) located on the human chromosome 17p13.1 and has been involved in the organization of apoptosis, cell growth, nuclear factor-κB, and Ras [9-10].

1.3 The structure of TNK1

The structure of a catalytic domain can be divided into large and small segments, as ATP binds to protein substrate. Upon binding of substrate to nRTKs and ATP, catalysis of phosphate transfer happen in a cleft between these two segments. It was found that nRTKs have some sequence predilection about the target Tyr. For instance, the Src preferred sequence is Ile/Val–Tyr–Gly–Val–Leu/Val and Glu–Glu/Asp–Ile–Tyr–Gly/Glu–Glu–Phe [11]. Various preferred sequences about Abl and Tyr in Src suggest that these two types of nRTKs phosphorylates different targets. One of the proteins (protein-protein) interaction domains in nRTKs are the 3 SH3 (SRC Homology 3 Domain) domains and the Src homology 2 SH2 (SRC Homology 2 Domain) [12]. The longer the SH2 domain (~100 remains) binds the phosphotyrosine (P-Tyr) remains in a sequence-specific manner. The SH3 domain is smaller (~60 remains) and bind proline-containing sequences capable of forming a poly-proline kind II helix. Some Non-receptor tyrosine kinases without SH2 and SH3 domains possess same subfamily-specific domains that is used for proteins (protein-protein) interaction. TNK1b consists of 8 exons encoding a 47-KDa protein, which we initially detected in differentiating murine embryonic stem cells and named it Kos1 (kinase of embryonic stem cells) [13]. Kos1 contributes the NH2 terminal 380 amino acids, including the entire kinase domain with TNK1a and includes a unique 54 amino acid series at its COOH terminal. TNK1/Kos1 belongs to the Ack family of NRPTKs. But, different from Ack1 and Ack2 [14-15], TNK1/Kos1 lacks a CRIB motivation that can bind the GTP-bound form of stimulating cdc42. The antisera elevated against a peptide from the NH2 terminal precinct of TNK1/Kos1 discovered only a 47-KDa product in cell lines (including human) and other tissues that were construed, indicating that the 47-KDa Kos1 may be the dominant protein expressed in mice [13]. TNK1/Kos1 expression is developmentally organized in mouse embryos, as long as in differentiating embryonic stem cells in vitro [13]. Imposed expression of TNK1/Kos1 in different cell lines inhibits cell growth via a mechanism requiring its substantial tyrosine kinase activity [10-13]. As shown that in (Fig. 1).

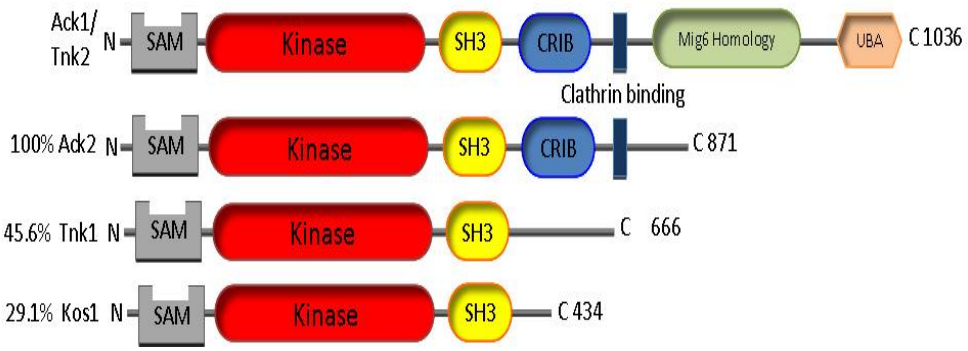

Fig. 1. The structure of TNK1**.** Consist of Ack1-TNK2, Ack2, TNK1, Kos1, SH3 domains.

1.4 The functions of TNK1

The main function of nRTKs is participating in signal transduction in activated T-cells (thymus) and B-cells (bone marrow) in the immune system [16]. Signalling by many receptors is dependent on nRTKs contains B-cell receptors (BCR), T-cell receptors (TCR), interleukin-2 receptor (IL-2R), Ig receptors, erythropoietin and prolactin receptors (EpoR). CD4 (cluster of differentiation 4) and CD8 (cluster of differentiation 8) receptors on T-lymphocytes require their signaling. When antigen binds to T-cell receptor, becomes autophosphorylated and phosphorylates a zeta chain of the T-cell receptor, thereafter another nRTK, binds to this T-cell receptor and is then involved in the downstream signalling proceedings which mediate transcriptional activation of cytokine genes. which leads to the phosphorylation and recruitment of nRTK. Another nRTK, is also implicated in signalling mediated via the B-cell receptor. Mutations are accountable for X-linked agammaglobulinemia [17-18], a disease characterized via the shortage of mature B-cells.

II. Materials and Methods

2.1 Reagents, antibodies and others

- DMEM Medium (from HyClone Labs, USA)
- -0.25% Trypsin (from Gibco, USA)
- FBS (Fetal Bovine Serum from Gibco, USA)
- DMSO ((Dimethyl sulfoxide from Sigma, USA)
- -G418
- -Penicillin / streptomycin
- PVDF membrane (polyvinylidene fluoride from Millipore Immobion, USA)
- RIPA buffer (Beyotime, China)
- Primary antibody Beta-actin (Beverly, MA, USA)
- Primary antibody TNK1 (tyrosine non receptor kinases) (Beverly, MA, USA)
- Primary antibody Vimentin (Beverly, MA, USA)
- Primary antibody Beta-catenin (Beverly, MA, USA)
- Secondary antibody, anti-mouse and anti-rabbit HRP-conjugated antibodies were purchased from Cell Signalling Technology (Beverly, MA, USA)
- X-ray film (Kodak, USA)
- Cell culture plates 10cm and 3.5cm (Thermo Scientific, China.)

2.2 Apparatus

- Cell culture incubator (Thermo scientific, Model 311, USA)
- Advanced Research Microscope (Model Nikon Eclipse 80i, Japan)
- Inverted microscope (Olympus, Japan)
- Electrophoresis and membrane transfer apparatus (Biorad, USA)
- Refrigerators (Electrolux, France)
- -80 ^{o}C and -20 ^{o}C freezers (Hair, China
- Sonicator (Scientz, Model Jy92-II, USA)
- Centrifuge (Eppendorf, Model 5810k, USA)
- Nitrogen Tank (Thermo scientific, Model 8147, USA)
- Heating block (Mixing Black Model MB-102, Bioer, China)
- Ice-maker (XUEKE, China)

2.3 Cells and cell line

PC-3 human prostate cancer cell lines PC-3 human prostate cancer cell lines are one of the cell lines utilized in the research of prostate cancer. These cells are helpful in the investigation of biochemical change in prostate cancer cells and in evaluating their response to chemotherapeutic factors. Furthermore, they have been used to create cancers under dermis in the mice for the aim to get a model of the cancer environment in the framework of the organism.

Description: PC-3, DU145 and LNCaP cells are the most commonly used prostate cancer cell lines in the laboratory. PC-3 cells have high metastatic potential, while DU145 and LNCaP cells have moderate and low metastatic potential respectively [19]. The PC-3 cell line was created in 1979 from the bone metastasis of class IV prostate cancer in a 62-year-old Caucasian male. These cells do not respond to the androgen. PC-3 cells have been reduced in testosterone-5-alpha activity of the enzyme acid phosphatase [19]. Moreover, karyotype analysis showed that PC-3 cells are almost three-ploidy, and contain 62 chromosomes. They have common features of tumour cells with the epithelial origin, such as junction complexes, numerous microvilli, abnormal mitochondria, abnormal nuclei and nucleoli, annulate lamellae, and lipoidal bodies. As shown that in (Fig. 2).

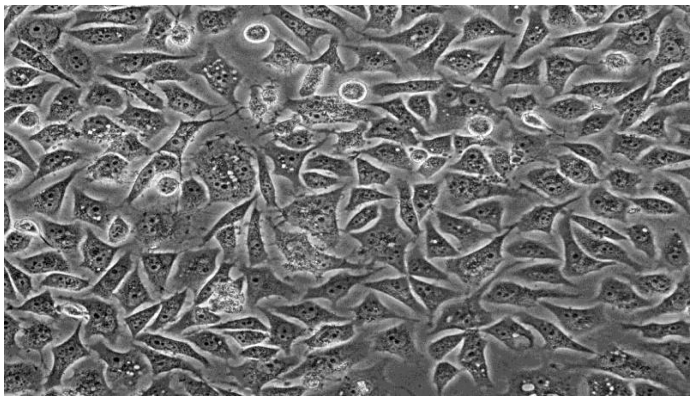

Fig. 2. Morphology of PC-3 cells. The image was obtained from website https://commons. wikimedia.org/wiki/File: PC-3-cell.jpg

2.4 MTT assay

PC-3 cells with confluence around 95 % were washed with 1X PBS and trypsinized with 0.25% Trypsin, then centrifuged at 125 g for 5 minutes. The supernatant was discarded and the cell pellet was re-suspended in 1-10 ml of fresh medium. To count the cell number, 100 µl of cell suspension was mixed with 900 µl of DMEM, and the cells were counted under the microscope. Cell number was calculated according to the formula: average cell number × dilution number $\times 10^4$. 2×10^4 cells were seeded into 24-well plates and maintained in the incubator at 37 °C without CO_2 for 1-4 days. The medium was replaced after 48 hours. Each cell clone was tested in quadruplicate.

At the end of each day, the MTT solution (0.5 mg/ml) prepared with Opti-MEM® Reduced-Serum Medium was added to each well and incubated in the incubator for 45 minutes, then discard MTT solution, 500 µl of DMSO was added and the plate was gently shaken for 10 minutes protected from light. Finally, Optical Density (O.D.) was read at 490 nm and 560 nm. The viability of the cells was determined by the following formula:

$$Cell\ viability\ (\%) = \frac{O.D\ value\ of\ treated\ well - O.D.value\ of\ the\ blank}{O.D.value\ of\ control\ well - O.D.value\ of\ the\ blank} \times 100$$

2.5 Clonogenic assay

Cell culture, trypsinization, centrifugation and cell counting are similar to MTT assay, but the cells were diluted as shown in the following diagram. As shown that in (Fig. 3).

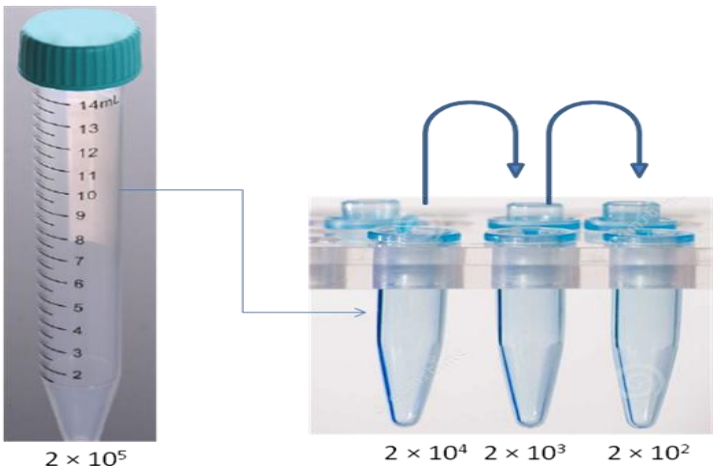

Fig. 3. 1 ml of the cell suspension (200 cells / ml) was added to the 24-well plates and maintained in the incubator for 10 days at 37 °C without CO_2. The cells were fixed by adding 500 µl Methanol to the each well for 15 minutes, and then stained with 1% Crystal Violet in 20% methanol 100µl for 20 minutes. Finally, the plate was washed with water gently and observed under microscope at 10X magnification.

2.6 SDS-PAGE

2.6.1 Preparation of gel solutions

8 % separating gel was prepared as shown in Table.

Table 1. Recipe for 8% SDS-PAGE stacking gel (10 ml)

Reagents	Volume (ml)
ddH_2O	4.6
30 % acrylamyde	2.7
1.5 M Tris buffer (pH 8.8)	2.5
10 % SDS	0.1
10 % AP	0.1
TEMED	0.006

5 % separating gel was prepared as shown in Table.

Table 2. Recipe for 5% separating gel solution (6ml)

Reagents	Volume (ml)
ddH_2O	4.1
30% acrylamyde	1.0
1.5 M Tris buffer (pH 8.8)	6.8
10% SDS	0.6
10% AP	0.6
TEMED	0.006

2.6.2 Protein separation

To cast 1.5 mm thickness of the gel, put the long plate to the short plate and fixed together in the gel matrix. 7 ml to the 8% concentration of the gel solution was added between the plate in the matrix gel and add the isopropanol for the surface of the gel, then keep it for one hour in the room temperature and make sure do not move it, and the gel was washed with distilled water. And then to prepare the upper gel 2.5 ml of the concentration of the gel was added into the first gel, and put the comb on top of the concentration of the gel, then keep it for half hour in the room temperature and make sure do not move it. When the gel will be ready, the gel plates take off from the shelf, and then put the gel plate into the electrophoresis tank that containing 1X electrophoresis buffer. Equal amounts of cell lysis to load into the gel, and the electrophoresis be run at 80 V, Am more than 300 for 10 minutes to make sample level equal, and then follow by 110 V, Am more than 300 for 2 hrs to separate proteins.

2.7 Membrane transfer

2.7.1 Buffer preparation

10X transfer buffer preparation:

Table 3. 10X Transfer Buffer (1L)

Reagents	Weight (g)	Final concentration (M)
Trizma base	30.3	0.25
Glycine	144	1.92

The chemicals dissolve in distilled water to a final volume of 1L, pH 8.3.

1 X transfer buffer preparation:

Table 4. 1X Transfer Buffer (1L)

Reagents	Volume (ml)
Methanol	200
10X transfer buffer	100
ddH_2O	700

2.7.2 Membrane transfer

After electrophoresis, have been separated gel plates, and the concentration of the gel was decreased. The bottom right-hand corner of the gel was blunted for orientation to carry the gel from the plate or glass, and then soak the gel in the transfer buffer. At the same time, cut a piece of PVDF membrane convenient to the size of the gel and soak in transfer buffer. Then put the gel in the transfer cassette. To put the transfer cassette in the electrophoresis tank containing 1X membrane transfer buffer. And then put the tank in the ice box, and the membrane transfer was running at 80 V, Am more than 300 for 10 minutes to make sample level equal at the gel convenient with the membrane, and then follow by 110 V, Am more than 300 for 2 hrs.

2.8 Wester blot

2.8.1 Buffer preparation

1. Tris-buffered saline (10X TBS)

Table 5. 10X TBS Buffer (2L) pH 7.6

Reagents	Weight (g)	Final concentration (mM)
Trisbase	48.4	10
NaCl	160	150

The reagents are dissolved in 2 L-distilled water and pH 7.5 with HCl (hydrochloride) to increase PH, and NaoH (Sodium hydroxide) to decrease pH.
2. TBST buffer: 900 ml 10X TBS buffer with 1 ml Tween-20 (1X TBS buffer containing 0.05% of Tween-20).
3. 5% blocking buffer (5g to 100 ml) of BSA or nonfat dry milk are dissolved in TBST buffer, stored at 4°C.
4. Primary antibody diluent buffer 5%: 0.5g of BSA is dissolved in 100ml TBST buffer, stored at 4°C.
5. Secondary antibody diluent buffer 5%: 0.5g of nonfat dry milk is dissolved in 100 ml TBST buffer, stored at 4°C.

2.8.2 Wester blot

At the end of the transfer, after finish the transmembrane process takes the membrane from the cassette, put into a small container, and then wash with 10 ml of TBST buffer for a short time (15 minutes). The container was then placed on the shaker and block the membrane with 5% blocking buffer (5g to 100 ml) of BSA or nonfat dry milk are dissolved in TBST buffer at room temperature for at least 2 hours. Then wash the membrane for half hour three times with TBST buffer at room temperature. To investigate for the protein, incubate the membranes with primary antibodies (1:2000 dilution) overnight at 4°C plus to the movement. The next day, wash the membrane for 1 hour three times (10 mins each) with TBST buffer at room temperature and incubate with secondary antibody (1:20000 dilution) at room temperature for 2 hrs plus to the movement on the shaker. After widely washing for 1 hour three times (10 mins each) with TBST buffer at room temperature, put the membrane in the autoradiography cassette, exposed to X-film, and developed in the dark room.

III. Results

3.1 TNK1 deficiency inhibited cell survival

TNK1 deficient cell clones have been established in our laboratory by using CRISPR-Cas9 technology. In this study, TNK1 C37, TNK1 C43, and TNK1 C1 indicate TNK1 deficient cell clones 37, 43, and C1 respectively. The pX330 C1 indicates empty vector transfected clone C1.

To investigate the effect of TNK1 on cell survival, MTT was performed. The results showed that TNK1 deficiency significantly decreased cell survival. As shown in (Fig. 4) the cell survival of TNK1 C37, TNK1 C43, and TNK1 C1 decreased 43.35%, 65.74%, and 67.03% respectively compared to pX330 C1. The deficiency of TNK1 was confirmed by Western blot.

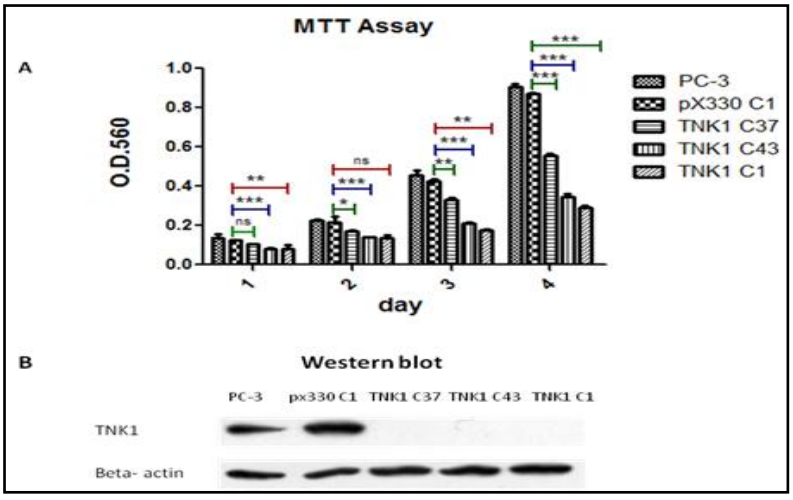

Fig. 4. TNK1 deficiency reduced cell survival of PC-3 cells. After cells reach 95% confluence in 10 cm plates, cells were trypsinized, 2×10^4 cells were seeded into 24-well plates and incubated for 1-4 days. MTT assay was performed to examine cell survival each day. **(A).** MTT assay results. The experiments were preformed three times and the results were reproducible. ***P<0.001, **P<0.01, *P<0.05,ns< not significant. **(B).** The TNK1 level in different cell clones as assessed by Western blot. Beta-actin was used as internal control.

3.2 TNK1 deficiency inhibited clonogenicity

MTT assay results indicate that TNK1 promoted cell survival. To identify whether TNK1 is involved in cell proliferation, clonogenic assay was performed. The results showed that TNK1 deficiency not only

dereased the number of cell clones but also the size of clones (Fig. 5), suggesting that TNK1 promotes cell survival and proliferation.

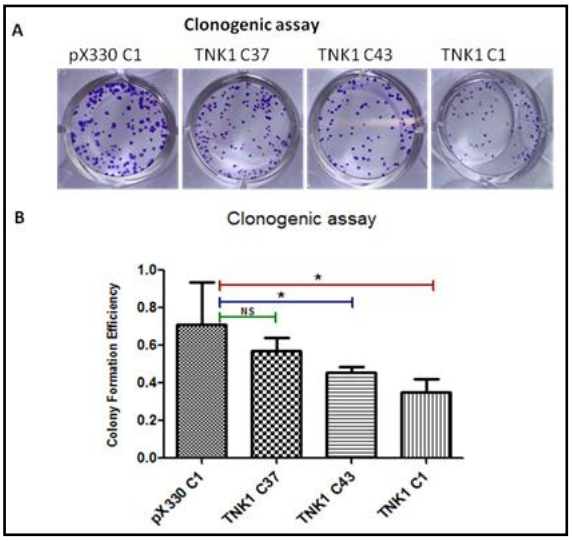

Fig. 5. TNK1 deficiency inhibited of cell survival in the clonogenic assay. After cells reach 95% confluence in 10 cm plates, cells were trypsinized, and 2×10^4 cells were seeded into 24-well plates, then incubated for 10 days. Clonogenic assay was performed to examine cell survival each day. **(A).** Clonogenic assay results. Images one represents wells of each clone that was taken. **(B).** Quantitative analysis of clonogenic assay. The experiments were preformed three times and the results were reproducible. ***P<0.001, **P<0.01, *P<0.05, ns<not.

3.3 The impact of TNK1 on the expression of EMT markers

According to the literature, the decreased expression of epithelial marker and increased expression of mesenchymal markers beta-catenin and vimentin is a good feature of the carcinoma [20], also featuring which enhances the metastatic and invasive characteristics of the PC-3 cell line. The effect of TNK1 on the expression of EMT markers was examined, the results showed that (Fig. 6).

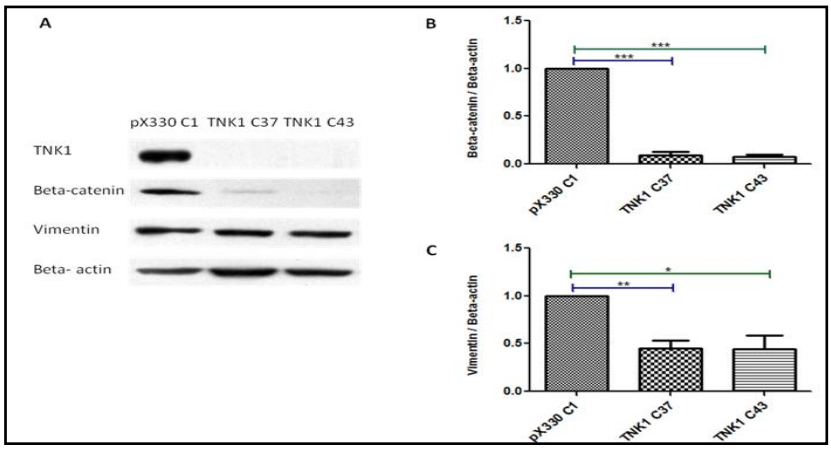

Fig. 6. TNK1 deficiency effects expression levels of EMT markers. After cells reach 95% confluence in 10 cm plates, cells were trypsinized, and quantified the TNK1 protein level with the lysis and loading buffer. The cell lysate samples were resolved by SDS-PAGE followed by membrane transfer. Loaded cells volumes around 20 µl cells and blotting. The experiments were preformed two times and the results were reproducible. The amount of protein was determined using ImageJ® programme. **(A).** Western blot results. **(B).** Quantitative analysis of Beta-catenin/Beta-actin. **(C).** Quantitative analysis of Vimentin/Beta-actin. The experiments were preformed three times and the results were reproducible. ***P<0.001, **P<0.01, *P<0.05, ns<not.

IV. Discussion

In 2011, Meredith C. Henderson and colleagues used HT-RNAi screening to identify kinases whose silencing decreased the growth of pancreatic cancer cells. Furthermore, they validated the kinase TNK1 as a novel pancreatic cancer target that is important in cell growth and survival [21]. TNK1 plays a critical role in cancer as it is demonstrated in previous studies that it could inhibit cell growth and cell survival. In vitro, the percentage of cells are low in the clones relative to normal cells and cell control for MTT assay and Clonogenic assay. TNK1 deficiency enhanced expression of mesenchymal cell marker beta-catenin and vimentin.

In this study, where the used cell number 2×10^4 cells, increased the rate growth of the cells in the MTT assay, and also that happened with clonogenic assay, where 2×10^4 used which also increased the growth rate of the cells, that the percentage of cells are low in the clones relative to normal cells and control cell for MTT assay and Clonogenic assay, compared with the control in this test. TNK1 enhanced expression of the epithelial cell marker molecule and suppressed Mesenchymal cell marker molecule Beta-catenin and Vimentin.

References

[1]. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray, F. Cancer Incidence and Mortality Worldwide: IARC Cancer Base GLOBOCAN. 2012, 1, 11.

[2]. José Expósito, Isabel Linares, Isabel Castillo, Miguel Martínez, Pilar Vargas, Ismael Herruzo, José Antonio Medina, Amalia Palacios, Eloísa Bayo, Francisco Peracaula, Javier Jaén, José Antonio Sánchez, and María José Ortiz. Evaluation of the utilization of external radiotherapy in the treatment of localized prostate cancer in Andalusia, Spain. Radiat Oncol. 2015, 10, 265.

[3]. Chamie K, William SB, Hu JC. Population-based assessment of determining treatments for prostate cancer. JAMA Oncol. 2015, 1, 60-67.

[4]. Heidenreich A1, Bastian PJ2, Bellmunt J3, Bolla M4, Joniau S5, van der Kwast T6, Mason M7, Matveev V8, Wiegel T9, Zattoni F10, Mottet N11; European Association of Urology. Guidelines on prostate cancer. Arnhem (The Netherlands): European Association of Urology (EAU). 2014, 65, 467-479.

[5]. Male Genitals - Prostate Neoplasms". Pathology study images. University of Virginia School of Medicine. 2011. http://www.med-ed.virginia.edu/courses/path/gu/prostate3.cfm

[6]. Franklin RB, Milon B, Feng P, Costello LC. Zinc and zinc transporter in normal prostate function and the pathogenesis of prostate cancer. Frontiers in Bioscience. 2005, 10, 2230-9.

[7]. Robinson DR, Wu YM, Lin SF. The protein tyrosine kinase family of the human genome. Oncogene. 2000, 19, 5548-57.

[8]. Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S. The protein kinase complement of the human genome. Science (New York)**.** NY. 2002, 298, 1912–1934.

[9]. Hoehn GT, Stokland T, Amin S. Tnk1: a novel intracellular tyrosine kinase gene isolated from human umbilical cord blood CD34+/Lin-/CD38- stem/progenitor cells. Oncogene 1996, 12, 903–913.

[10]. Azoitei N, Brey A, Busch T, Fulda S, Adler G, Seufferlein T. Thirty-eight-negative kinase1 (TNK1) facilitates TNFα-induced apoptosis by blocking NF-Kb activation. Oncogene 2007, 26, 6536–45.

[11]. Songyang Z, Carraway KL, Eck MJ, Harrison SC, Feldman RA, Mohammadi M, Schlessinger J, Hubbard SR, Smith DP, Eng C. "Catalytic specificity of protein-tyrosine kinases is critical for selective signalling". Nature. 1995, 373, 5369.

[12]. Kuriyan J, Cowburn D. "Modular peptide recognition domains in eukaryotic signalling". Annu Rev Biophys Biomol Struct. 1997, 26, 259-288.

[13]. Hoare K, Hoare S, Smith OM, Kalmaz G, Small D, May WS. Kos1, a nonreceptor tyrosine kinase that suppresses Ras signalling. Oncogene 2003, 22, 3562–77.

[14]. Manser E, Leung T, Salihuddin H, Tan L, Lim L. A non-receptor tyrosine kinase that inhibits the GTPase activity of p21cdc42. Nature. 1993, 363, 364–367.

[15]. Yang W, Cerione RA. Cloning and characterization of a novel Cdc42-associated tyrosine kinase, ACK-2, from bovine brain. J Biol Chem. 1997, 272, 24819–24.

[16]. Weiss A, Littman DR. "Signal transduction by lymphocyte antigen receptors". Cell. 1994, 76, 263–274.

[17]. M Vihinen, D Vetrie, H S Maniar, H D Ochs, Q Zhu, I Vorechovský, A D Webster, L D Notarangelo, L Nilsson, and J M Sowadski. Structural basis for chromosome X-linked agammaglobulinemia: a tyrosine kinase disease. 1994, 91, 12803-12807.

[18]. Tsukada S, Saffran DC, Rawlings DJ, Parolini O, Allen RC, Klisak I, Sparkes RS, Kubagawa H, Mohandas T, Quan S. "Deficient expression of a B cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia". Cell. 1993, 72, 279-290.

[19]. Kaighn, M.E.; K.S. Narayan; Y. Ohnuki; J. F. Lechner; L.W. Jones. "Establishment and characterization of a human prostatic carcinoma cell line (PC-3).". Invest Urol. 1979, 17, 16-23.

[20]. Michael Zeisberg1, Eric G. Neilson2. Biomarkers for epithelial-mesenchymal transitions. J Clin Invest. 2009, 119, 1429–1437.

[21]. Meredith C. Henderson,1,2 Irma M. Gonzales,1,2 Shilpi Arora,2,3 Ashish Choudhary,2 Jeffrey M. Trent,4 Daniel D. Von Hoff,1 Spyro Mousses,2 and David O. Azorsa1,2. High-throughput RNAi screening identifies a role for TNK1 in growth and survival of pancreatic cancer cells. Mol Cancer Res. 2011, 9, 724–732.