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New Bio-Therapeutic Candidate for Pancreatic Cancer

Marwah Saad Joudah[1], Basma Talib Al-Sudani[2], Inam Sameh Arif[1]

[1]College of Pharmacy, Branch of Toxocology, Mustansiriyah University, Iraq,
[2] College of Pharmacy, Branch of Clinical Laboratory Sciences, Mustansiriyah University, Iraq

Abstract

SIRT1 is NAD^+-deacetylases, several histone and non-histone proteins which their involvement in metabolic processes, cell growth, apoptosis, and senescence are well known. Several Sirtuins targets are implicated in cancer. SIRT1 is both an oncogene and tumour suppressor, and it can act in this capacity depending on the kind of tissue and the cancer etiology. Subsequent studies in this field are going to make evident the exact function of SIRT1 at the cancer site and it is hoped that new chemotherapeutic functions of SIRT1 activators are going to be determined. In accordance with this, it is suggested that very selective ligands such as aptamer was created and investigated in various pancreatic cancer cell lines for the regulated activity of SIRT1. This study seeks to establish the therapeutic impact of the activator SIRT1 aptamer as a pharmacological model of pancreatic cancer by evaluating the impacts of activators SIRT1 (aptamer) on the growth of a series of human pancreatic cancer cell lines (AsPC-1, Capan-2, BxPC-3). Results gotten from *in vitro* cytotoxicity assays revealed that circular aptamer BAS inhibited the growth of pancreatic cancer cell lines [BxPc-3 (80%), Capan-2 (83%) and Aspc-1 (82.8%)] at 72h with IC_{50} 0.55, 0.5, 0.76 µM respectively. Importantly, circular aptamer showed no reduction of cell viability on the non- pancreatic cancerous H6c7 cell line, implying it might be safe to non-cancerous tissue. Our results indicate that the SIRT1 activated by aptamer could a promising targeted therapeutic approach for pancreatic cancer.

Keywords: *aptamer, pancreatic cancer, SIRT1.*

Introduction

Pancreatic cancer is considered as a deadly tumor with no improvement in its prognosis regardless of the recent progress in cancer interventions. Many of the interventions for pancreatic cancers are associated with disappointing results. Though curative surgery is associated great benefits, it is hard to achieve in most advanced pancreatic cancers. Interventions such as conventional chemotherapy, radiation therapy, and even targeted chemotherapy have shown disappointing results. Therapies for target tumors by using monoclonal antibodies are the most thriving cancer therapies [1]; however, there are different limiting factors. These limiting factors include high costs and low penetration to tissue [2]. There is a need for the development of more efficacious and cost-effective targeted interventions. Aptamer (ssDNA, RNA or peptide oligomers) have been extensively studied for their therapeutic properties [3]. Al –Sudani, 2017 was selected a circular aptamer against SIRT1 as used to treated many of cancer cell by activation of SIRT1 [4]. Deacetylation of histones and non-histones proteins is by SIRT1. These histones and non-histone proteins are implicated in a variety of biochemical activities. These biochemical activities include metabolism, cell growth, apoptosis, and senescence [5].The function of SIRT1 in tumorigenesis and cancer advancement is not fully understood but it was stated that its function could be tissue-type and context specific [6]. Pancreatic adenocarcinoma up-regulated factor (PAUF) is known to induce a swift spread of pancreatic cells by up-regulating b-catenin. This is based on the postulation that b-catenin could be a target molecule for pancreatic cancer therapy with a speculation that whether there is SIRT1 suppression of b-catenin in pancreatic cancer cells that express PAUF (Panc-PAUF). It is suggested that evaluation of such suppression could result in inhibition of the proliferation of these cells. The amounts of b-catenin protein and its transcriptional role in Panc-PAUF cells are (SIRT1 activator) siRNA improves b-catenin expression and transcriptional activity. Through the down-regulation of cylin-D1, activation of SIRT1 leads to inhibition of the growth of Panc-PAUF cells. Cyclin-D1 is a

target molecule of b-catenin. [7]. This project aimed to evaluate the therapeutic effect of the activator SIRT1 aptamer (BAS aptamer) as a pharmacological model for pancreatic cancer cells.

Methodology

Cell culture

Human pancreatic cancer cells: AsPC-1, Capan-2, and BxPC-3 were gotten from American Type Culture Collection ATCC (Middlesex, UK). Human pancreatic normal cells: H6c7 were bought from Kerafast (Boston, USA).Capan-2 cells was preserved in McCoy's 5A Medium, AsPC-1 and BxPC-3 cells were stabilized in Roswell Park Memorial Institute-1640 (RPMI-1640) medium, all media were enhanced with 10% fetal bovine serum FBS and 1% L Glutamine as well as to 1% Penicillin-Streptomycin-Amphotericin B 100X as antiseptic. H6c7 cells were maintained in Keratinocyte SFM + EGF + bovine pituitary extract (Invitrogen). Then they were supplemented with 1x antibiotic-antimycotic (Gibco). Cells were cultured in 75cm^2 flasks and incubation carried out in 5% CO2/95% humidified air at 37°C. Once the cells reached 90% confluency, flasks containing Capan-2, BxPC-3, and AsPC-1 were kept under sterile conditions. The cells were subjected to washing with 5 ml of phosphate buffered saline solution (PBS) and then incubate for 2 min in trypsin solution at 37°C to permit cells to separate from the bottom of the flask. Addition of an equal volume of complete growth media was done, and the cell suspension was moved into a 50ml conical tube. Centrifugation of the cells was performed at 1200 rpm for 3 min. Separation of the supernatant was done, and the cell pellet underwent re-suspension in fresh supplemented growth media. Cells were counted under the microscope on a haemocytometer and use as required. The cells were stored at -80 °C for 24hrs and then were stored under liquid nitrogen.

Cell viability by MTT assay

The MTT assay was used assessing the effects of aptamer on pancreatic cancer cell viability. A 100 µl from all cells suspensions (Capan-2, BxPC-3, AsPC-1 and H6c7) were dispense into 96-well flat-bottom tissue culture plates at concentrations of 5 x 10^{3}cells per well and were incubated 24h under standard conditions; 4 x 10^3 cells/well for 48h incubation, and 3 x 10^3 cells/well for 72h incubation. After 24 h, the cells would have treated with 2.5 µM circular aptamer (BAS). After a recovery period 24h, 48h and 72h, the cell culture medium was removed and cultures was incubated with medium containing 30 µl of MTT solution (3 mg/ml MTT in PBS) (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) for 4h at 37°C. After 4h this medium was done with by gentle inversion and tapping onto paper. Control wells receive only 100 µl growth media. 100 µl of dimethyl sulfoxide (DMSO) was added to all wells, the plates were then kept at room temperature in the dark for about 15-20 min. The absorbance of each well was measured by multi-scan reader at a wavelength of 540 nm and correcting for background absorbance using a wavelength of 650 nm. IC_{50} was evaluated by MTT assay at 72h after the cells exposure to aptamer. The concentration range used for circular aptamer (BAS) are, 0.0078, 0.0156, 0.0312, 0.625, 0.125, 0.25, 0.5, 1.00 µM.

Immunofluorescence microscopy

The seeding of the cells was performed at roughly 10,000 cells per well in 96-well clear bottom imaging tissue culture plates. Eighteen hours later, cells were treated with 1µM circular aptamer (BAS) and incubation done in 5% CO2/95% humidified air at 37°C overnight, after that the washing of the cells was done thrice in PBS, fixing for 5mins at room temperature with Formalin 4%, washing 2 times in PBS, then will be permeabilised by 0.5% Triton X-100 for 5mins, washing 3 times with PBS; nonspecific binding was obsructed with 3% FBS for 1h at room temperature, then the blocking solution was removed, followed by an addition of 100 µl/well of Anti-Sirtuin1 antibody to the cells. After that incubation was done overnight at 4°C in a wet tray. The following day, cells will be washed thrice in PBS, and incubated for 2h in dark at room temperature with 1:2000 Alexa Fluor 546 goat anti-mouse IgG (AF546)/1% FBS in PBS. Thereafter the washing of the cells was done thrice in PBS. At the end, the preparation was treated with 10 µl mounting medium containing DAPI for staining cell nuclei. It was left for 1hr before the microscopic examination using fluorescence microscopy

Results

Cell viability by MTT assay

The cell viability of circular aptamer, at 2.5µM concentration shows it is extremely active on the different pancreatic cancer cells up to a period of 72 hours. As demonstrated in figure 1, BxPc-3,Capan-2 and Aspc-1 cells demonstrated to be highly sensitive with an increase response dependent on time (cell death around

80%, 83% and 82.8% at 72h, respectively). Importantly, circular aptamer showed no reduction of cell viability on the non-cancerous H6c7, implying it might be safe to non-cancerous tissue. The IC50 of circular aptamer was very low concentration (0.55, 0.5, 0.76 µM) in BxPc-3, Capan-2, and Aspc-1 cells respectively.

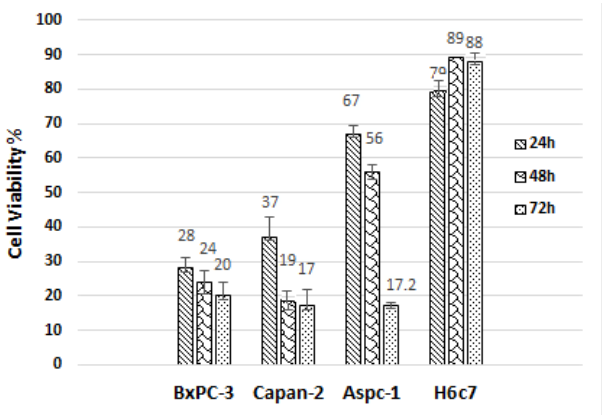

Figure 1: Cell viability studies on different pancreatic cancer cell lines, at 24, 48 and 72h of incubation. All samples were run in triplicate, control is expressed as 100% viable cells. Cells were challenged with circular aptamer at 2.5µM. At 24h the cell viability was as: 79%, 28, 37, and 67 for Aspc-1, BxPc-3, Capan-2 and H6c7 respectively. At 48h the cell viability was as: 89%, 24, 19, and 56 for Aspc-1, BxPc-3, Capan-2, and H6c7 respectively. At 72h the cell viability was as: 88%, 20, 17, and 17.2 for Aspc-1, BxPc-3, Capan-2, and H6c7 respectively.

Determine the Location of Circular Aptamer by Fluorescence Microscopy

To determine the location of the circular aptamer, fluorescence microscopy was used in this experiment. The circular aptamer was labelled with green fluorescent, DAPI (4', 6-Diamidino-2-Phenylindole, Dihydrochloride) was utilized for locating the nuclei and a Texas Red (sulfonyl chloride) antibody for SIRT1. The cells were dosed with circular aptamer at a low concentration to avoid cell death depending on their IC_{50}. Thus, figure 2 showed that fluorescence microscopy is very useful tool for studying the location of circular aptamer in cancer and non-cancer cell lines. Cells treated with circular aptamer were brightly fluorescent and over-expression of SIRT1 as showed in figures 2. In contrast, there was no observable fluorescence signal from control H6c7 cells treated with the circular aptamer.

As a figure 2 shown the localization of SIRT1 in BxPc-3 and Capan-2 cells in the cytoplasm of these cells, while in AsPC-1 and H6c7cells, the SIRT1 was localized in the nucleus. These results strongly suggested that this aptamer is highly affinity and selectivity binding with SIRT1 as the results of fluorescent imaging shown that when the aptamer present in this cancer cells it could be activated and increased the expression of the SIRT1 enzyme.

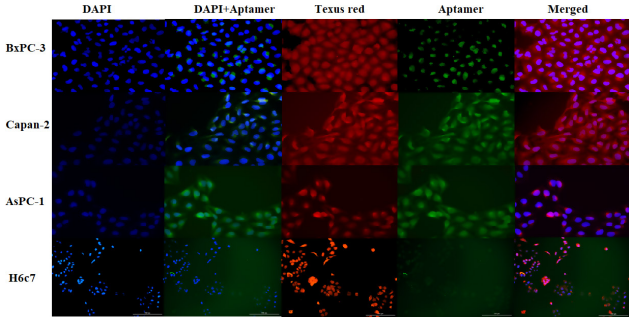

Figure 2: Fluorescence microscopy analysis of *circular* aptamer binding to SIRT1 enzyme in BxPC-3, Capan-2, AsPC-1 and H6c7 cell lines. *Cells* pretreated with *1* µM *circular* aptamer. C*ircular* aptamer was labelled with green fluorescent, nuclei were stained with DAPI (Blue) and a Texas Red antibody for SIRT1 (Red). Fluorescence intensity were measured by luminometer microplate Readers, fluorescence measurement system with an excitation wavelength of 395, 359 and 596 nm and an emission wavelength of 509, 461 and 615 nm of GFP, DAPI and Texas Red respectively. Images were taken at a magnification of 100X.

Discussion

This study investigated oligonucleotide aptamer and its usefulness for anticancer drug discovery. Our study has provided information concerning the usage of MTT assay in assessing the inhibitory effect of SIRT1-aptamers on viability of BxPc-3, Capan-2, and Aspc-1 cell lines and non-cancer cells (H6c7). Our experiments using Aspc-1, BxPc-3, and Capan-2 cancer cell lines demonstrates that circular aptamer BAS has a growth suppressor characteristics. Kabra, (2010) was found that knockdown of SIRT1 raises the percentage of tumour advance by promoting cell spread, whereas over-expression of SIRT1 decreases tumour induction and expansion in nude mice [8]. This result is in agreement with our suggestion that the pharmacological activation of SIRT1 by aptamer causes reduction of the rate of cell viability in Aspc-1, BxPc-3, and Capan-2 cell lines as shown in figure 1. Our results indicate that SIRT1 works as a context-dependent tumour suppressor. Also activation of SIRT1 causes suppression of tumour initiation and promotion of cell death. The anti-apoptotic role of SIRT1 is well known and it is rooted in the assumption that SIRT1 acts as an oncogene. However, it was revealed that transgenic mice over-expressing SIRT1 decreased the progress of neoplasia in the intestine caused by Apc^{Min} mutation, this suggests a tumour inhibitory function of SIRT1 [9]. Many studies have pointed out that

SIRT1 could behave as an oncogene. This is possible because of the higher association of normal expression of SIRT1 level in specific tumors than normal tissue [10]. In contrast, Kabra, (2010) established that SIRT1 levels are uneven in various phases of colon cancer tumours. It has been reported that SIRT1 has both oncogenic and tumour-suppressive functions. The expression of SIRT1 at high levels shows that SIRT1 is effective in inducing G1 arrest [8]. Indeed, our results confirmed these previous suggestions that activators of SIRT1 by circular aptamer could have therapeutic potential as an anti-cancer target. Another condition is that activators of SIRT1 could affect cancer blocking impacts by promoting the growth-inhibitory impact of SIRT1 in cancer cell line, and revealed the decrease level of intracellular ROS production. Through direct deacetylation the inactivation of the p65 subunit of NF-κB is caused by SIRT1. Inhibition of NF-κB causes suppression of the iNOS (inducible nitric oxide synthase are a family of enzymes catalysing the production of nitric oxide (NO) from L-arginine) and thus could decrease the cellular ROS load [11]. To advance our study the role of SIRT1, circular aptamer was used to study the activity of SIRT1 in (BxPc-3, Capan-2, and Aspc-1) cells. The results observed in this study are consistent with several research groups have reported that resveratrol which activator SIRT1 was inhibited much more cancer cells proliferation such as Caco-2 [12], MCF-7, A549 [13], U2OS ([14], MDA-MB-468 [15] and HepG2 [16]. Interestingly, it has been demonstrated that there is not effect to used SIRT1-aptamers on H6c7 cells viability, which indicating that circular aptamer have actively killing cancer cells without affecting normal cells. The IC_{50} of circular aptamer was very low concentration (0.55, 0.5, 0.76 μM) in BxPc-3, Capan-2, and Aspc-1 cells respectively. This low concentration of IC_{50} is very good results because the low dose of circular aptamer can suppress the spread of cancer cells. To study the localization of SIRT1 in these cells, fluorescence microscopy was used and the results were showed the different location of SIRT1 between cytoplasm and nuclei depending on the type of cells. As a figure 5 shown the localization of SIRT1 in BxPc-3 and Capan-2 cells in the cytoplasm of these cells, while in AsPC-1 and H6c7cells, the SIRT1 was localized in the nucleus.

In Conclusion, the results provided here indicate that circular aptamer could be effective in treating pancreatic cancer because its properties of a growth suppressor for specific killing of the tumor cells only avoiding unpleasant side effects from damage to the rest of the body. Additional research works are required, for instance, the cellular mechanisms of action of SIRT1 in aptamer-mediated apoptosis still needs a lot of study.

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Conflict of Interest - None

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