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Study on Effect of Buqi-Tongluo-Jiedu Decoction on Protein Concentration of HIC1/SIRT1 of Pancreatic Cancer Model Mice

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Received Date: 21 March 2014
Accepted Date: 27 June 2014
Published Date: 30 June 2014

Citation: Bo H, You WC, Sheng CX (2014) Study on Effect of Buqi-Tongluo-Jiedu Decoction on Protein Concentration of HIC1/SIRT1 of Pancreatic Cancer Model Mice. Enliven: Challenges Cancer Detect Ther 1(1): 005.

Abstract

Objective: To observe the effect of protein concentration of HIC1/SIRT1 on pancreatic cancer model mice.

Method: 30 mice were randomly selected and divided into three groups, each group comprised 10 mice. Cancer cells were injected into model mice. Normal group and model group were given saline. The treatment group was given Buqi-Tongluo-Jiedu decoction. After 14 days, after administering euthanasia the tumor tissues were removed. To detect HIC1/SIRT1 protein concentration, the specimens of each group were examined by WB method.

Result: There is no significant difference between of the treatment group and of the model group in the HIC1 protein concentration, $P>0.05$. But there is a significant difference compared with that of the normal group, $P<0.05$. The protein concentration of SIRT1 of the treatment group was significantly lower than the model group, $P<0.05$, but the protein concentration of SIRT1 of the treatment group was significantly higher than the model group i.e., $P<0.05$.

Conclusion: Buqi-Tongluo-Jiedu decoction plays a regulatory role on HIC1/SIRT1 protein concentration of the model mice.

Keywords: The model mice; Pancreatic Cancer; HIC1/SIRT1 protein

Introduction

Pancreatic cancer is a highly malignant tumor and lack effective treatment. Modern research shows that HIC1/SIRT1 compound can inhibit the Transcription by binding to promoter of SIRT1 [1]. So, HIC1 and SIRT1 constitute a signal transduction pathway. It plays an important role in the pathogenesis of malignant tumors.

According to Professor Qiu Xingfan,"ZhangXue LuoBi DuJie" of cancer pathogenesis, combined with Clinical applications, establish Buqi-Tongluo-Jiedu prescription to treat pancreatic cancer, has obtained the good clinical curative effect [2]. In order to further explore molecular biology mechanism and its effects, Buqi-Tongluo-Jiedu prescription was applied on pancreatic cancer model mice, to observe the HIC1 / SIRT1 protein concentration of specimen, so as to provide experimental basis of Buqi-Tongluo-Jiedu prescription treating pancreatic cancer.

Materials and Model

Laboratory animal

SPF male Nude mice (BALB/C) 30, 6-8 weeks, weight 20±2g, from HuaZhong University of Science and Technology, Tongji medical college.

Cell line

Human pancreatic cancer SUIT-2 from Peking union medical college, Institute of Oncology.

Cell culture

Human pancreatic cancer cell line SUIT-2 recovery, Add RPMI1640 culture medium containing 10% fetal calf serum, placed in 37°C, 5% CO_2, humidified CO_2 incubator culture.

Establishment of animal models

A week ahead of the experiment it was observed , if Nude mice grow normally (no more than 10% mortality) ,to establish xenograft models, Mice were injected SUIT-2 cells 1×106 in the lateral cutaneous nerve of left hind leg in the model group, meanwhile saline was injected into the normal group on lateral cutaneous nerve of the left hind leg. After the modeling, the model group were randomly divided into the model group and the treatment group (N=10).

Drugs and preparation

Buqi-Tongluo-Jiedu prescription:

(ginseng 5g, radix astragali 30g, fructus aurantii immaturus 10g ,rhizoma chuanxiong ,15 g, earthworm 10g, radix bupleuri 8g, the centipede 3g, rhizoma zedoariae 15g, morel 15g, roasted liquorice 6g, etc.).

Themouseequivalentdoseperkilogramofbodyweightis60gcrudedrugaccording to Human and experimental animal body surface area conversion method.

The herbs were soaked in cold water, five times volume of herbs for 20 minutes, and decoction was left to boil on high flame and then on low flame for 40min, and the liquid was extracted by filtration. Later cold water was added to herbs and allowed it to boil, on low flame for 30 minutes, and dregs filtered out of the decoction, after blending two Pharmaceutical extracts and concentrated into Pharmaceutical extract with every milliliter containing crude drug of 2 g, it was stored in the refrigerator from 0 to 4°C.

Dose and method of administration

Normal group

3ml/100g weight given with normal saline, once a day, for 14 days.

Control group

After transplantation of cancer cells and transplanted tumor visible, 3ml/100g weight was given with normal saline, once a day, for 14 days.

Treatment group

After transplantation of cancer cells and transplanted tumor visible, 3ml/100g weight was given once a day, for 14 days.

Drawing materials

After treatment, Mice were administered cervical dislocation euthanasia and operating parts of the skin was disinfected with alcohol. Mice were placed in a clean bench, tumor nodules was striped and stored at 20°C in the refrigerator.

Statistical analysis

Spss10.0 package was used for data processing, P <0.05 was recognized as having significant difference

Instruments and reagents used for analysis

UV spectrophotometer	752-P
Electronic balance	PL-203
Tabletop centrifuge	TGL-16c
Refrigerated centrifuge	15R heal force
DBRO-SYS	AJC-0501-P
Magnetic stirrer	79-1
Shaker	WD-9405A
Electrophoresis System	DYY-6C
water bath kettle	TL-420D
Refrigerator	BCD-186
SDS-PAGE gel preparation kit	Google biological company
Total cell protein extraction kit	Google biological company
ACTIN	SC-1616R SANTA
GAPDH	SANTA
Tubulin	SANTA
Histone H3	SANTA
50*cooktail	Roche
PMSF (100mM	Google biological company
Phosphorylation of protease inhibitors	Google biological company
5×SDS-PAGE loading buffer	Google biological company
ponceau dye solution	Google biological company
Coomassie brilliant blue G - 250 dye solution	Google biological company
ECL solution	Google biological company
Film	Kodak company
Tween-20	0777 Amresco
HRP mark goat resistance of rabbit antibody	KPL
HRP mark rabbit anti goats antibody	KPL
HRP mark rabbit anti mice antibody	KPL
HRP mark rabbit political rat antibody	KPL
BSA	Roche
PVDF membrane (0.45um)	IPVH00010 Millipore
Protein marker (10-170 kDa)	sm0671 Ferments
Actin	Sc-1616r Santa
SIRT1-抗	#2496 CST
HIC1-抗	Sc-271499 Santa

Experimental Methods

Tissue protein extraction

Organization

Tissue block was washed 2-3 times with cold TBS, to remove blood if any and cut into small pieces and put in the slurry apparatus. Add 10 times the volume organization volume this reagent (within minutes before use to join cooktail + phosphorylation protease inhibitor), slurry thoroughly in ice. If need to improve protein concentration, we can reduce the reagent volume appropriately. Homogenate was transferred to 1.5 ml centrifuge tube for oscillation incubated put on ice bath for 30 min, pipette was used to beat upon repeatedly, to ensure cell cracking completely. Later it was centrifuged for 5 min in 12000 g, supernatant was collected, namely the total protein solution. (Supplementary Files)

Determination of protein concentration (Bradford method)

- Standard curve production.
- 10 mg/ml BSA to 1 mg/ml was diluted with physiological saline.
- Sample concentration measurement.
- 1 µl measure protein and 99 µl 0.9% saline was added to 900 µl Bradford, after blending them, absorbency was tested in the 595nm. According to standard curves, the sample protein concentration was calculated
- After measuring the protein content, the volume containing 40 µ g protein solution was calculated, for the sample quantity.
- To the protein samples, appropriate volume of 5 × protein sample buffer was added. This was placed in boiling water bath for 5 minutes.

SDS-PAGE electrophoresis

- Glass plate was cleansed.
- Compound and the sample were encapsulated.
- Aligned glass was placed into the folder lock, in operation, two glass alignments made, and adhesive was applied to prevent leakage.
- According to the experimental arrangement, separation glue was prepared, after TEMED was added, shook immediately and the gap was filled with glue. After about 45 min, glue was removed by rinsing upper layer with water and residue was dried off with water absorbent paper.
- Concentrated gum was filled in the opening space between the glass plates and then comb was inserted in the concentrated glue.
- Enough electrophoresis fluid was added, sample was used to electrophoresis
- Sample concentrated gum was added into electrophoresis hole (tank?), at 75 v voltage, separation glue with 120v. when bromophenol blue just ran out, power supply was terminate the electrophoresis, turn membrane.

Turn (Transfer to a suitable membrane?) membrane

- Six 7×9cm filter paper were pre cut a modest 0.45 um PVDF membrane was used, before using PVDF membrane was activated by dipping in methanol.
- Transfer apparatus was set up for transferring membrane i.e., fluid basin, two pieces of rubber cushion, a glass rod, filter paper and activated PVDF membrane.
- Open clamp, and make black side keep level. mat sponge On the mat, three layer filter paper.
- Gel was carefully separated, and covered with filter paper. Membrane was covered with glue, and bubbles removed. The membrane was covered with three filter paper and the air bubbles removed. Finally was covered with another rubber cushion.
- Turn (conditions for transfer?) conditions 200m A1 hour.

Immune response

- The membrane was blocked in 5% of the skim milk (0.5% TBST match) for 1 hour, in room temperature condition and using decolorizing table.
- The first antibody was diluted (TBS - T dissolved 5% skim milk), and incubated at 4°C for the night.
- It was washed 3 times with TBST in room temperature condition and on decolorizing table, each time for 5 minutes
- The second antibody was diluted to 3000 times with TBST, and incubated for 30 minute in room temperature condition, was washed 3 times with TBST in room temperature condition and on decolorizing table, each time for 5 minutes

Chemiluminescence

- A and B two medium volume reagents were mixed in a centrifugal tube, the membrane protein facing up and fully in contact with this mixture, after 1-2 minutes, residual fluid was removed , wrapped and put in X - ray clip to exposure.
- Exposure conditions were adjusted according to the different light intensity.

Gel image analysis

The film was scanned and archived, Alpha software processing system analyze light density value of target band, regarding normal group gray value as 1.00 ± 0.00, other groups sample value was the ratio value of the normal group.

Result

Group	Animal Number	HIC1	SIRT1
The normal group	10	1.00±0.00★	1.00±0.00■
The model group	10	0.64±0.45▽	4.17±2.03▼
The treatmen group	10	0.60±0.15▽●	2.58±1.09▽★

Table1 Specimens HIC1/SIRT1 protein concetration gray value注：▽Compared with the normal group P<0.05, ▼Compared with the normal group P<0.01, ▼Compared with the normal group P>0.05, ★Compared with model group P<0.05, ▲Compared with model group P<0.01, ●Compared with model group P>0.05.

Discussion

Domestic and international study found that the HIC1 and target genes HiRE binding can regulate transcription of target genes. There are two HiRE in SIRT1 promoter region, Inhibition of its transcriptional activity can be inhibited by the complexes of SIRT1 protein and HIC1. HIC1 may be combined to the HiRE of FGF-BP1promoter region. It plays an important role in the inhibition of tumor angiogenesis [3]. HIC1 and SIRT1 constitute a signaling pathway.

According to Professor Qiu Xingfan, (authors guide) "ZhangXue LuoBi DuJie" of cancer pathogenesis [4], combined with Clinical applications, establish Buqi-Tongluo-Jiedu prescription. in prescription, Ginseng is sweet, little bitter, little warm, reinforcing vital energy, invigorating the spleen and benefiting the lung, as monarch drug. Astragalus is sweet, little warm, replenishing qi and nourishing yang, tonifying qi and strengthening exterior, enhancing the efficacy of ginseng, as the official drug. Radix bupleuri and poncirus are pungent., warm, promoting the circulation of qi, ligusticum and aromatic turmeric are pungent ,warm, promoting blood circulation for removing blood stasis, as adjuvant. Earthworm and centipede are pungent warm dredging meridians, ablation of communications, as conductant drug. Black nightshade .is cold, bitter, little sweet, clearing away the heat evil and expelling superficial evils, as adjuvant. Prepared radix glycyrrhizae is sweet, little warm, coordinating the drug actions of a prescription as conductant drug. The prescription has effect of promoting qi circulation to remove meridian obstruction, resolving toxin and dispersing tumor.

The results shows HIC1 protein concentration: the normal group was highest, model group protein concentration was significantly lower than that of the normal group, and there was a significant difference between the normal group and the model group.

Modeling success: The HIC1 gene transcription ability was down and HIC1 protein synthesis decreased, therefore the organization HIC1 protein concentration decreased. HIC1 protein concentration of the treatment group and the model group had no significant difference; however, there was significant difference between the HIC1 protein concentration of the treatment group and that of the normal group. This phenomenon shows though the HIC1 protein concentration did not return to normal, the treatment prevents its further decline.

This may be related experimental sensitivity or therapeutic time too short, The reason needs further study SIRT1 protein concentration: The model group was highest, while the normal the lowest, The SIRT1 protein concentration of the normal group was significantly lower than the model group. The SIRT1 protein concentration of the treatment group was significantly lower than that of the model group, but still significantly higher than the normal group. This phenomenon shows SIRT1 gene expression was elevated and the SIRT protein synthesis increased in pancreatic cancer cell. Therefore the Organization SIRT proteins concentration increased. This phenomenon show SIRT1 gene expression is down, but still did not recover to normal levels. Whether the treatment effect increased with time, it remains in further observation.

Reference

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