



# Effect of taurine on the proliferation, apoptosis and *MST1/Hippo* signaling in prostate cancer cells

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**Background:** To investigate the effects of taurine on prostate cancer cell proliferation and apoptosis, and on the mammalian sterilization of a 20-like kinase-1 (*MST1/Hippo*) signaling pathway.

**Methods:** The prostate cancer DU145 cell line was selected and the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method was used to determine the rate of inhibition by taurine on the proliferation of the cells at 1, 10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-3</sup>, 10<sup>-4</sup>, 10<sup>-5</sup> and 10<sup>-6</sup> mg/mL to obtain the taurine intervention concentrations. The cultured cells were divided into three groups: the blank group was cultured with conventional culture medium, the positive control group was cultured with 2 mg/mL cisplatin, and the taurine group was cultured with the determined taurine intervention concentrations of 0.003 mg/mL as low, 0.03 mg/mL as medium and 0.3 mg/mL as high concentration. After 72 h incubation, cell proliferation, apoptosis and cellular *MST1/Hippo* signaling pathway protein expression were observed.

**Results:** In the comparison of cell proliferation rate, the taurine group was lower than the positive control group and the blank group (P<0.05), the cell proliferation rate of different concentrations in the taurine group decreased with the increase of concentration (P<0.05). The apoptosis rate was higher in the taurine group than in the positive control group and the blank group (P<0.05), the apoptosis rate increased with increasing concentration in the taurine group (P<0.05). The expression of *MST1* and *Bax* was higher in the taurine group than in the positive control group and the blank group (P<0.05), the expression of *MST1* and *Bax* increased with increasing concentration in the taurine group (P<0.05). The expression of *YAP* and *Bcl-2* was lower in the taurine group than in the positive control group and the blank group (P<0.05), the expression of *YAP* and *Bcl-2* decreased with increasing concentration in the taurine group (P<0.05).

**Conclusions:** Taurine promoted apoptosis and inhibited proliferation of prostate cancer cells, and its mechanism of action may be related to the *MST1/Hippo* signaling pathway in a dose-dependent manner.

**Keywords:** Apoptosis; cell proliferation; mammalian sterilization of a 20-like kinase-1/*Hippo* signaling pathway (*MST1/Hippo* signaling pathway); prostate cancer; taurine

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## Introduction

Prostate cancer is among the most common malignancies of the male urinary tract. The latest epidemiological survey data from the International Agency for Research on Cancer in 2018 showed that prostate cancer accounted for 13.5% of all new cases of malignancies in men worldwide, with some patients dying from complications related to prostate cancer, which made it the 5th leading cause of cancer death in men worldwide (1). With increasing clinical research on prostate cancer, it has now been found that its development is associated with a variety of signaling abnormalities, among which mammalian sterilization of a 20-like kinase-1 (*MST1*) is aberrantly expressed in prostate cancer, and the *Hippo* signaling pathway to which *MST1* belongs can induce the development of this disease (2). Although the mechanism of prostate cancer was identified, clinical treatment is still based on early surgery, radiotherapy and chemotherapy, which can control progression of the disease in the short term, but the 5-year survival rate of patients is still low. Taurine is a protective substance with multiple effects, and is thought to inhibit inflammatory responses, resist oxidative stress-induced damage and regulate immune disorders, as well as promoting apoptosis of cancer cells. Studies (3,4) have shown that taurine can inhibit tumor cell proliferation, promote apoptosis and induce autophagy. The PI3K/Akt/mTOR pathway is a central regulatory mechanism that regulates normal cell physiology and also plays a role in tumor genesis and metastasis. The inhibition of the PI3K/Akt/mTOR pathway can activate the autophagy of prostate cancer cells. *MST1* plays a role in regulating cell cycle, promoting apoptosis and inhibiting tumor growth. Taurine is a sulfur-containing amino acid with simple chemical structure and is also a conditionally essential nutrient for human body. Taurine has a wide range of physiological effects, is the body's endogenous anti-injury substance. In recent years, it has been found that taurine has important application value in the prediction, prevention and treatment of tumors, and the concentration and transport of taurine *in vivo* are closely related to the occurrence and development of tumors. This study explored the mechanism of taurine in prostate cancer and provided important guidance for the clinical treatment of prostate cancer patients. Taurine, known chemically as 2-aminotaurine, is a sulfur-containing amino acid found in the body that has a simple chemical structure. The results of xenograft experiment in nude mice showed that the expression of anti-apoptotic protein Bcl-2 was significantly

decreased and the expression of pro-apoptotic protein *Bax* was significantly increased after taurine treatment.

In this context, we selected a prostate cancer cell line and used a taurine intervention to analyze its effects on prostate cancer cell proliferation, apoptosis and the *MST1/Hippo* signaling pathway to provide a reference for subsequent clinical studies. We present the following article in accordance with the MDAR reporting checklist (available at <https://tcr.amegroups.com/article/view/10.21037/tcr-22-1297/rc>).

## Methods

### Materials

The prostate cancer DU145 cell line was provided by Utilico (Shanghai) Life Sciences Ltd. (item No. YLK-XB127). The suppliers of the main reagents were: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent (Nanjing KGI, China); taurine (Merck Sigma, USA); *MST1* antibody, *YAP* antibody, *Bax* antibody, *Bcl-2* antibody, horseradish peroxidase (HRP)-labeled secondary antibody (Abcam, USA).

### Cell culture

The prostate cancer DU145 cells were cultured in Dulbecco's modified Eagle medium (DMEM) at 37 °C and trypsinized when the cells were 90% fused, after which they were passaged and logarithmically grown cells were harvested for subsequent analysis.

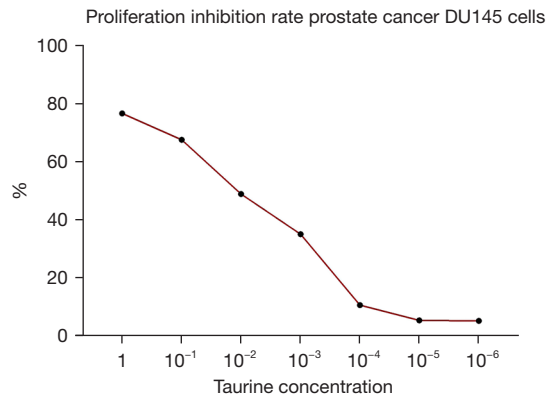
### Analysis of taurine intervention concentrations

The rate of inhibition by taurine on the proliferation of prostate cancer DU145 cells at concentrations of 1, 10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-3</sup>, 10<sup>-4</sup>, 10<sup>-5</sup> and 10<sup>-6</sup> mg/mL was determined using the MTT method. The cell concentration was adjusted to 3×10<sup>4</sup> cells/mL, and the suspension was prepared after the adjustment. Cells were inoculated in 96-well plates and incubated at 37 °C for 72 h, after which MTT reagent was added to obtain the cell optical density (OD) values and analyze the rate of inhibition by taurine: inhibition rate = (1 - OD value of taurine intervention wells)/OD value of blank wells × 100%. A quantitative-effect relationship curve was obtained and the taurine intervention concentration was determined after the calculation of the median lethal concentration

**Table 1** Effect of taurine on the proliferation of prostate cancer DU145 cells

Taurine concentration (mg/mL)	OD value, $\bar{x} \pm s$	Inhibition rate (%)
1	0.17±0.04	76.54
10 <sup>-1</sup>	0.19±0.03	67.43
10 <sup>-2</sup>	0.23±0.04	48.82
10 <sup>-3</sup>	0.29±0.05	34.96
10 <sup>-4</sup>	0.47±0.07	10.58
10 <sup>-5</sup>	0.45±0.05	5.28
10 <sup>-6</sup>	0.43±0.04	5.11

OD, optical density;  $\bar{x} \pm s$ , mean  $\pm$  standard deviation.

**Figure 1** Taurine dose-effect relationship curve.

(LC50) value for taurine was completed.

### Grouping and intervention

The prostate cancer DU145 cells were divided into three groups: the blank group was cultured using conventional culture medium, the positive control group was cultured with 2 mg/mL cisplatin, and the taurine group was cultured using taurine intervention concentrations of 0.003 mg/mL as low, 0.03 mg/mL as medium and 0.3 mg/mL as high. The effect on prostate cancer DU145 cells at each concentration was observed after 72 h of culture.

### Cell proliferation assay

Cells from each group were harvested and the cell proliferation rate was determined using the MTT method:

cell proliferation rate = (OD of the cell group tested/OD of the control group)  $\times$  100%.

### Measurement of apoptosis

Cells from each group were harvested and the apoptotic rate was analyzed using the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) method: cells were washed, stained using 4',6-diamidino-2-phenylindole (DAPI), blocked and the nuclei color was observed using fluorescence microscopy, where green represents positive and blue represents negative. Apoptosis rate = number of positives/total cells  $\times$  100%.

### MST1/Hippo signaling pathway protein assay

Cells from each group were harvested and analyzed for MST1/Hippo signaling pathway protein expression using western blot: 50  $\mu$ g of the prepared protein was extracted and added to 2 $\times$  SDS buffer, and after electrophoresis, membrane transfer, mold taking, fixation and closure, the MST1, YAP, Bax and Bcl-2 primary antibodies (1:1,000 TBST dilution) were incubated at 4  $^{\circ}$ C, washed with TBST after 24h, HRP-labelled secondary antibody was added, the membrane was washed, and DAB was used as an internal reference to obtain MST1, YAP, Bax, Bcl-2 protein expressions.

### Statistical analysis

The SPSS 26.0 statistical package was used. Measurement data are described as mean  $\pm$  standard deviation ( $\bar{x} \pm s$ ), analysis of variance was used for comparison among multiple groups, an independent sample *t*-test was used for comparison between groups, and  $P < 0.05$  was considered as a statistically significant difference.

## Results

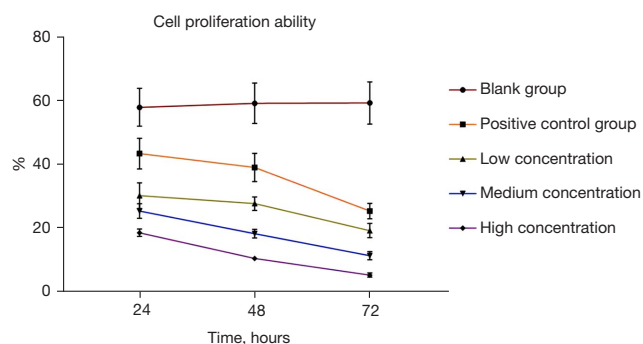
### Taurine LC50 values, and experimental group determination

As shown in Table 1 and Figure 1, MTT analysis found that the rate of inhibition by taurine on proliferation of prostate cancer DU145 cells gradually decreased with each concentration, and its LC50 value was calculated as 0.3156 mg/mL. The dose-effect relationship curve was plotted, which showed that, combined with the LC50 value,

**Table 2** Comparison of the proliferative capacity of cells in each group

Group	Cell proliferation capacity (%)		
	24 h	48 h	72 h
Blank group, $\bar{x}\pm s$	57.65±5.90	58.93±6.32	59.03±6.57
Positive control group, $\bar{x}\pm s$	43.21±4.76*	38.92±4.39*	25.31±2.39*
Taurine group, $\bar{x}\pm s$			
Low concentration	30.11±3.98 <sup>#</sup>	27.60±2.10 <sup>#</sup>	19.19±2.21 <sup>#</sup>
Medium concentration	25.31±2.26 <sup>#</sup> <sup>▲</sup>	18.21±1.34 <sup>#</sup> <sup>▲</sup>	11.37±1.28 <sup>#</sup> <sup>▲</sup>
High concentration	18.53±1.15 <sup>#</sup> <sup>▲</sup> <sup>Δ</sup>	10.48±0.69 <sup>#</sup> <sup>▲</sup> <sup>Δ</sup>	5.32±0.65 <sup>#</sup> <sup>▲</sup> <sup>Δ</sup>

\*P<0.05; compared with the blank group, compared with the positive control group, <sup>#</sup>P<0.05; compared with the low-concentration taurine group, <sup>▲</sup>P<0.05; compared with the medium-concentration taurine group, <sup>Δ</sup>P<0.05.  $\bar{x}\pm s$ , mean  $\pm$  standard deviation.

**Figure 2** Trend of cell proliferation capacity in each group.**Table 3** Comparison of the apoptotic capacity of each group

Group	Apoptosis rate (%)
Blank group, $\bar{x}\pm s$	7.43±1.12
Positive control group, $\bar{x}\pm s$	16.94±2.97*
Taurine group, $\bar{x}\pm s$	
Low concentration	32.54±4.34 <sup>#</sup>
Medium concentration	45.97±5.31 <sup>#</sup> <sup>▲</sup>
High concentration	55.58±6.43 <sup>#</sup> <sup>▲</sup> <sup>Δ</sup>

\*P<0.05; compared with the blank group, compared with the positive control group, <sup>#</sup>P<0.05; compared with the low-concentration taurine group, <sup>▲</sup>P<0.05; compared with the medium-concentration taurine group, <sup>Δ</sup>P<0.05.  $\bar{x}\pm s$ , mean  $\pm$  standard deviation.

the inhibition of prostate cancer DU145 cells was more significant at the low, medium and high concentrations of taurine set in this study as 0.003 mg/mL, 0.03 mg/mL and

0.3 mg/mL, respectively.

#### Comparison of cell proliferation capacity between groups

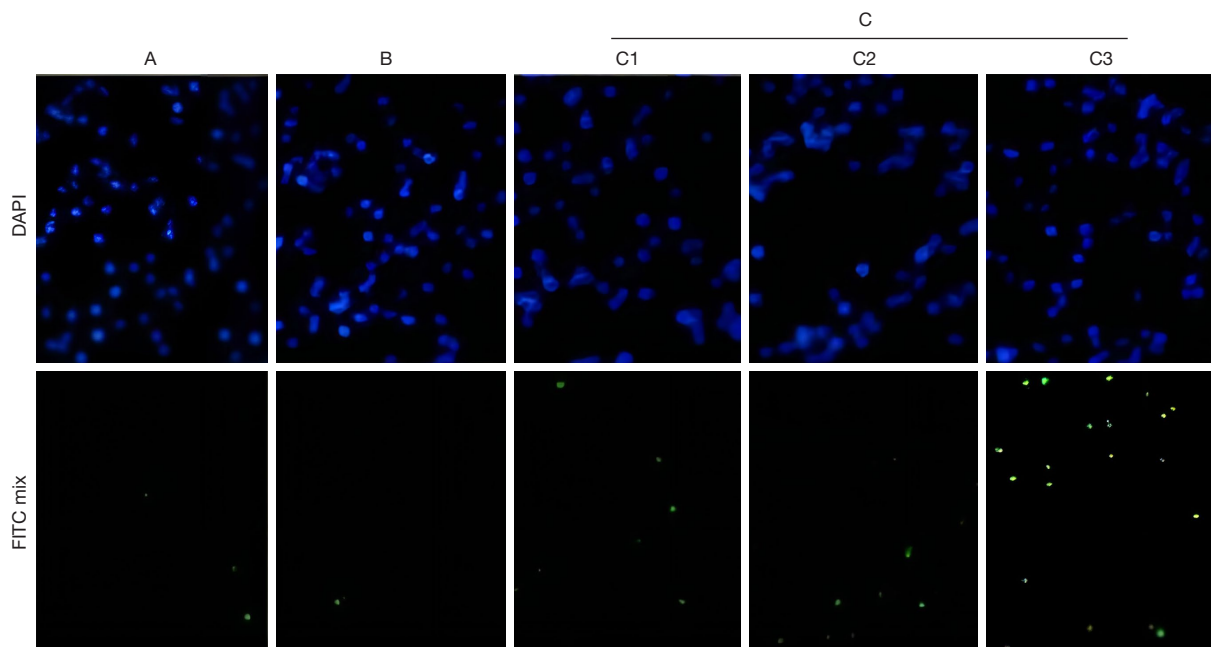
As shown in *Table 2* and *Figure 2*, the cell proliferation rates at 24, 48 and 72 h were lower in the positive control and taurine groups than in the blank group (P<0.05); the taurine group was lower than the positive control group (P<0.05); and the high concentration in the taurine group was lower than the medium concentration than the low concentration (P<0.05).

#### Comparison of apoptotic capacity among groups

As shown in *Table 3* and *Figure 3*, the apoptotic capacity of the cells was higher in the positive control group and taurine group than in the blank group (P<0.05); the taurine group was higher than the positive control group (P<0.05); and with the high concentration in the taurine group, it was higher than with the medium or low concentration (P<0.05).

#### Comparison of MST1/Hippo signaling pathway proteins among groups

As shown in *Table 4* and *Figure 4*, in terms of *MST1* and *Bax* protein expressions, the positive control group and taurine group were higher than the blank group (P<0.05); the taurine group was higher than the positive control group (P<0.05); with the high concentration in the taurine group expression were higher than with the medium or low concentration (P<0.05); in terms of *YAP* and *Bcl-2* protein expressions, the positive control group and taurine group



**Figure 3** Apoptosis TUNEL plot (x40). (A) Blank group; (B) positive control group; (C) taurine group (C1: low concentration, C2: medium concentration, C3: high concentration). DAPI, 4',6-diamidino-2-phenylindole; FITC, fluorescein isothiocyanate; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling.

**Table 4** Comparison of *MST1/Hippo* signaling pathway proteins among the groups

Group	MST1	YAP	Bax	Bcl-2
Blank group, $\bar{x}\pm s$	0.21±0.04	1.32±0.16	0.20±0.03	1.26±0.13
Positive control group, $\bar{x}\pm s$	0.56±0.06*	0.99±0.11*	0.43±0.05*	1.00±0.10*
Taurine group, $\bar{x}\pm s$				
Low concentration	0.79±0.08 <sup>#</sup>	0.68±0.09 <sup>#</sup>	0.76±0.08 <sup>#</sup>	0.75±0.07 <sup>#</sup>
Medium concentration	1.34±0.11 <sup>*#</sup> <sup>▲</sup>	0.45±0.03 <sup>*#</sup> <sup>▲</sup>	1.12±0.14 <sup>*#</sup> <sup>▲</sup>	0.39±0.05 <sup>*#</sup> <sup>▲</sup>
High concentration	1.68±0.23 <sup>*#</sup> <sup>▲</sup> <sup>△</sup>	0.20±0.05 <sup>*#</sup> <sup>▲</sup> <sup>△</sup>	1.64±0.23 <sup>*#</sup> <sup>▲</sup> <sup>△</sup>	0.18±0.03 <sup>*#</sup> <sup>▲</sup> <sup>△</sup>

\*P<0.05; compared with blank group, compared with positive control group, <sup>#</sup>P<0.05; compared with low-concentration taurine group, <sup>▲</sup>P<0.05; compared with medium-concentration taurine group, <sup>△</sup>P<0.05. *MST1*, mammalian sterilization of a 20-like kinase-1;  $\bar{x}\pm s$ , mean ± standard deviation.

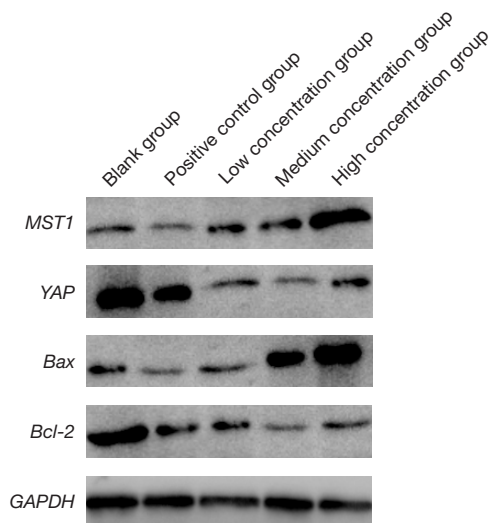
were lower than the blank group (P<0.05); the taurine group was lower than the positive control group (P<0.05); and with the medium concentration in the taurine group expression were lower than with the low concentration (P<0.05).

**Discussion**

The age group affected by prostate cancer is becoming

more and more youthful, and despite the current rapid advancements in medical technology, diagnostic tools and treatments that have achieved better results, the phenomenon of a low average survival period of patients still exists (5). Thus, it is particularly important to actively search for drugs that can effectively treat prostate cancer and prolong the life span.

Taurine is the main active ingredient in calculus bovis, which is mainly distributed in the reproductive



**Figure 4** *MST1/Hippo* signaling pathway proteins in each group. MST1, mammalian sterilization of a 20-like kinase-1.

organs, kidney, heart and brain of the human body, and is involved in a variety of physiological activities (6,7). It has been reported that taurine can regulate oxidative stress, suppress immune disorders and repair liver damage (8,9). Many clinical studies have been devoted to the role of taurine, such as Li *et al.*, who suggested that taurine induces apoptosis of cervical cancer cells (10), and He *et al.*, who also found that taurine induced apoptosis in cancer cells (11). Our results showed that the proliferation rate of cells cultured with taurine decreased and the apoptosis rate increased significantly, and the effect was strongest at high concentrations of taurine. These results suggested that taurine can dose-dependently inhibit the proliferation of prostate cancer cells and promote apoptosis, similar to the published results.

The *Hippo* signaling pathway is a recently discovered pathway that regulates cell growth. Currently, study is directed at the relationship between the *Hippo* signaling pathway and cell proliferation and apoptosis, and the pathway is believed to have a role in promoting lung cancer, prostate cancer, colorectal cancer and other tumors (12,13). The core kinase chain of the *Hippo* signaling pathway is *MST1*, with *YAP/TAZ* as the downstream target (14). When the *Hippo* signaling pathway is activated, *MST1* induces *YAP* phosphorylation in the presence of associated scaffolding proteins. When phosphorylated *YAP* accumulates in the cytoplasm, it is rapidly degraded by ubiquitination, thus

exerting its effect of inducing apoptosis and inhibiting cell proliferation (15-17). Wang *et al.* found that *FEAR1* and *FEAR4*-dependent *Hippo* pathway activation mediated DHA-induced apoptosis in androgen non-dependent prostate cancer cells (18). Salem *et al.* similarly found that the *Hippo* signaling pathway was involved in aberrant proliferation of prostate cancer cells (19). A previous study has found that *MST1* is highly conserved and its aberrant expression is associated with a variety of malignancies such as colorectal and hepatocellular carcinomas through its role in inducing cancer cell proliferation and apoptosis (20). *YAP*, on the other hand, is a candidate oncogene and is highly expressed in many types of human tumors (21). In the present study, we found that the expression of *MST1*, a key protein in the *MST1/Hippo* signaling pathway, was increased and the expression of *YAP* protein was decreased in the groups of cells with the taurine intervention at different concentrations.

Taurine can promote the secretion of pituitary hormone, activate the function of pancreas, so as to improve the state of the body's endocrine system, beneficial regulation of body metabolism, and has the function of promoting the organism immunity enhancement and anti-fatigue. Taurine can promote the recovery of acute hepatitis; Taurine had protective effect on the changes of rabbit primary renal tubular epithelial cells induced by cisplatin. Taurine has the function of inhibiting platelet agglutination, enhancing myocardial contractility and lowering blood pressure in blood circulation. Chemotherapy will have certain damage to the viscera, taurine can alleviate the damage caused by chemotherapy drugs. In addition, studies have found that taurine can inhibit cell proliferation and promote cell apoptosis in colorectal cancer, and taurine can inhibit cardiomyocyte apoptosis induced by adriamycin by up-regulating the activity of PI3K/Akt signaling pathway and down-regulating the activities of P53 and P38-JNK (22,23). Taurine can also induce autophagy and apoptosis of PC12 cells by activating mTOR signaling pathway and inhibiting methamphetamine.

In summary, taurine promoted apoptosis and inhibited proliferation of prostate cancer cells, and its mechanism of action may be related to the *MST1/Hippo* signaling pathway, acting in a dose-dependent manner. Although this study showed that taurine can inhibit the proliferation of prostate cancer cells, it needs to be further verified in patients with prostate cancer in order to improve the prognosis of prostate cancer patients.

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## Footnote

*Reporting Checklist:* The authors have completed the MDAR reporting checklist. Available at <https://tcr.amegroups.com/article/view/10.21037/tcr-22-1297/rc>

*Data Sharing Statement:* Available at <https://tcr.amegroups.com/article/view/10.21037/tcr-22-1297/dss>

*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at <https://tcr.amegroups.com/article/view/10.21037/tcr-22-1297/coif>). The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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