Hemin Downregulates the Effect of GPX4 on the Malignant Biological Behavior of Bladder Cancer T24 Cells

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Abastract: In a study of curcumin's effects on bladder cancer, T24 cells were used. Cells were divided into a control and 3 curcumin-treated groups (10, 20, 40 μmol/L). CCK8 showed curcumin inhibited proliferation in a time- and concentration-dependent manner. Flow cytometry showed increased apoptosis in curcumin-treated cells. Scratch test and Transwell assay indicated reduced migration and invasion. Western Blot revealed upregulation of HMOX1 and downregulation of GPX4 proteins in curcumin-treated groups ($P<0.05$). These findings suggest curcumin exerts antitumor effects by inhibiting proliferation, migration, invasion, and inducing apoptosis through downregulation of GPX4 pathway.

***Keywords*:** bladder cancer; malignant evolution; Hemin; GPX4

1. Introduction

Bladder cancer poses a serious threat due to its high recurrence and invasiveness. GPX4, an antioxidant enzyme, is abnormally expressed in cancers and may promote tumor development by regulating redox balance. Hemin, an iron-containing compound, enhances photodynamic therapy and anti-tumor immunity by inducing iron death via GPX4 downregulation and Fe2+ overload. This study aims to explore Hemin's effects on bladder cancer cells to reveal its therapeutic mechanisms and provide new strategies for bladder cancer treatment [1].

2. Data and methods

2.1 Data

T24 cells were purchased from Shanghai Cell Bank of CAS (No.: SCSP-536); Hemin from MCE (stock number: 16009-13-5); RPMI-1640 medium (stock number: 11875093) and fetal calf serum (stock number: A5669701) from Gibco; streptomycin (stock number: P1400), CCK-8 kit (stock number: CA1210) and crystal violet dye solution (stock number: G1062) from Beijing Treasure Technology Co., Ltd.; Matrigel Matrigel (stock No.: C0371), total RNA extraction kit (stock No.: R0016) and Annexin V-FITC / PI cell apoptosis detection kit (stock No.: C1062) were purchased from Shanghai Biyuntian Biotechnology Co., LTD.; HMOX 1 antibody (number: ab189491), GPX 4 antibody (number: ab125066) and GAPDH antibody (number: ab8245) were purchased from Shanghai Abcam.

2.2 Methods

(1) Cell Culture Cells were suspended in complete medium consisting of RPMI 1640 basal medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin mixture (100 U / mL penicillin and 100 μg/mL streptomycin). Cells were grown in a thermostatic cell incubator with 37 C, saturated humidity, 5% CO2.

(2) T24 cells in logarithmic growth were seeded at 5x10^5 cells/mL, 90 μL/well in a 96-well plate. Hemin solutions at 1, 5, 10, 20, and 40 μmol/L (in DMSO) were added to each well (6 wells/concentration). After 24 and 48h at 37°C, 5% CO_2, 10 μL CCK-8 was added and incubated for 4h. Absorbance was measured at 450 nm. Cell proliferation inhibition rate was calculated as [(Ac-As) / (Ac-Ab)]*100%, where Ab=blank, Ac=negative control, As=experimental. Experiments were repeated independently at least 3 times for reliability.

(3) T24 cells were seeded in 6-well plates and treated with 10, 20, or 40 μmol/L Hemin for 24h. Flow cytometry was used to detect apoptosis (early and late/necrosis) in ≥10,000 cells per group. Experiments were repeated three times with three biological replicates to ensure reliability.

(4) T24 cells (in the logarithmic growth phase) were seeded in a 6-well plate at a density of 5*104 cells per well. When cell confluence is about 80%, a 20 μ L sterile pipette. Three groups were low (10 μ mol/L), medium (20 μmol/L) and high (40 μmol/L) according to Hemin concentration. Photo cell migration was observed by using an inverted microscope at 0h

and 24h. Five visual fields were randomly selected and migration distances were calculated using ImageJ software analysis.

(5) Transwell After the diluted Matrigel matrix gel was evenly spread in the Transwell upper chamber and solidified, T24 cells were treated with Hemin at different concentrations (10,20 and 40 μmol/L). 100 μ L of cell suspension adjusted to 5*104 cells / mL was added to the upper chamber and serum-containing medium was added to the lower chamber and incubated for 48 hours. Cells were subsequently fixed with paraformaldehyde, stained with crystal violet, and five random fields were counted and photographed across the membrane under the microscope.

(6) T24 cells were divided into low (10 μmol/L), medium (20 μmol/L) and high (40 μmol/L) concentrations of Hemin. Hemin and drug solution were incubated for 24 hours. Prepare Matrigel matrix gel in a 24-well plate at 250 μL per well and stand for 1 hour. After drug-treated cells were collected, a single cell suspension was prepared and 500 μL of cell suspension was added to a 24-well plate to culture each well for 4 hours. Celcells were randomly selected under the microscope.

(7) T24 cells were divided into low, medium, and high Hemin groups (10, 20, 40 μmol/L). After 24h, total protein was extracted and analyzed by SDS-PAGE/Western blot with antibodies to HMOX1, GPX4, and GAPDH. Chemiluminescence revealed relative expression of target proteins normalized to GAPDH.

2.3 Evaluation index

The survival rate, apoptosis rate, cell scratch width, number of membrane cells and number of tubes, and the protein expression level of HMOX 1 and GPX 4 were compared in each group.

2.4 Statistical treatments

Data were analyzed using the GraphPad Prism 9.0 statistical software, first for normality using the Shapiro-Wilk test. If the data met normal distribution, t-test was used, one-way ANOVA was used for comparison between multiple groups; if the data did not meet normal distribution, rank sum test was used. A $P < 0.05$ was considered as having a statistically significant difference.

3. Results

(1) CCK-8 results showed that Hemin inhibited T24 cell proliferation in a concentration- and time-dependent manner, with increasing effects at 1-40 μmol/L after 24h and 48h. Based on this, Hemin was categorized into low (10 μmol/L), medium (20 μmol/L), and high (40 μmol/L) groups for further study.

(2) The flow cytometry results of Hemin-induced apoptosis in T24 cells showed that T 24 cells after 24 hours treatment at different concentrations (10,20,40 μ mol/L) were higher in the three drug concentration groups Significant increase in the untreated group [$(14.50 \pm 0.50)\%$, $(16.83 \pm 0.76)\%$, $(21.83 \pm 0.76)\%$ vs. $(11.83 \pm 0.76)\%$, respectively, all $P < 0.05$]. These results indicate that Hemin has a significant effect on apoptosis in T24 cells, and that this effect was enhanced with increasing drug concentration.

(3) The results of the scratch assay of Hemin on T24 cell migration showed that after 24 hours of Hemin treatment at different concentrations (10,20,40 μ mol/L), T24 cell migration increased significantly in the three untreated concentrations [(13.40 ± 0.95) um, (11.50 ± 0.50) um, (10.03 ± 0.31) um (15.23 ± 0.67) um, all $P < 0.05$). These results suggest that Hemin has a significant effect on T24 cell mobility, and that this effect is enhanced with increasing drug concentration.

(4) The results of T24 cell invasion experiment showed that after Hemin treatment for 24 hours at different concentrations (10,20,40 μ mol/L), the number of T24 cell invasion increased significantly in all the three drug concentration groups compared with the untreated group [(2013 ± 31), (1640 ± 100), (1095 ± 47) (2314 ± 81), respectively, all $P < 0.05$]. These results indicate that Hemin has a significant effect on the invasion number of T24 cells and that this effect was enhanced with increasing drug concentration.

(5) The results of tubule formation on T24 cells showed that after 24 hours of Hemin treatment (10,20,40 μ mol/L) increased significantly in the three drug concentration groups [(20366 ± 552.4) um, (15312 ± 945.4) um, (10875 ± 469.7) um (23483 ± 946.0) um, all $P < 0.05$]. These results indicate that Hemin has a significant effect on the angiogenic capacity of T24 cells, and that this effect is enhanced with increasing drug concentration.

(6) Western blot results indicate that after 24 hours of Hemin treatment at 10, 20, and 40 μmol/L, HMOX1 protein expression in T24 cells was significantly upregulated [(0.32 ± 0.03), (0.47 ± 0.03), (0.68 ± 0.02) vs. untreated (0.24 ± 0.05), all $P < 0.05$], while GPX4 protein expression was significantly downregulated [(0.58 ± 0.02), (0.44 ± 0.04), (0.32 ± 0.03) vs. untreated (0.87 ± 0.03), all $P < 0.05$]. These findings suggest that Hemin has a concentration-dependent effect on modulating HMOX1 and GPX4 protein expression in T24 cells.

4. Discussion

Despite the continuous progress of modern medical technology, the treatment of bladder cancer still faces many challenges. Recently, GPX 4 was found to overactivate in a variety of tumor cells, suggesting a potential important role in tumorigenesis and development.

Hemin, a natural breakdown product of hemoglobin, reduces GPX4 expression in bladder cancer T24 cells, inhibiting proliferation and inducing apoptosis. Its antitumor mechanism involves regulating GPX4 pathway. Hemin's advantages over traditional drugs include low toxicity and specificity in downregulating GPX4, minimizing damage to normal cells.

Although Hemin has demonstrated some potential in the treatment of bladder cancer, its clinical application still needs further investigation. First, large-scale clinical trials are needed to verify the efficacy and safety of Hemin in patients with bladder cancer. Second, further exploration of the mechanism of Hin, especially how it regulates GPX 4 expression to inhibit the malignant biological behavior of bladder cancer cells is needed.

In conclusion, the effect of GPX 4 on the malignant biological behavior of T24 cells in bladder cancer provides new ideas and targets for the treatment of bladder cancer, which has important research value and application prospects. Through continuous research and exploration, Hemin is expected to be a member of the treatment of bladder cancer, improving patient prognosis and improving their quality of survival.

References

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