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THE POTENTIAL OF

MOLECULAR HYDROGEN IN TREATING OVARIAN CANCER

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INTRODUCTION

One of the leading causes of cancer-related death among women is ovarian cancer. It has an insidious onset and there is a lack of early diagnosis of cancer symptoms. Ovarian cancer is classified into three types: epithelial ovarian carcinomas, germ cell tumors, and stromal cell tumors. The treatment for ovarian cancer entails a large amount of surgery followed by chemotherapy.

Despite carrying out intense research on how to get the best treatment done for ovarian cancer, its overall cure rate has been about 30% for the last 2 decades. Therefore, this led to the need to explore non-toxic and antitumor molecules for treating ovarian cancer.

One case study regarding the potential of molecular hydrogen in treating ovarian cancer was published in the Translational Cancer Research journal. Let us have a general discussion of this case study in detail:

Background

Molecular hydrogen (H2) is a physiologically inert gas. The biological relevance of this gas has come a long way since Ohsawa. It exerts a therapeutic antioxidant activity and prevents brain damage caused by ischemia and reperfusion. Scientists have reported that this gas has therapeutic effects in different diseases like neurodegeneration, ischemia-reperfusion (I/R) injuries, mitochondrial diseases, inflammation, metabolic syndrome, and cancer.

The possible effects of molecular hydrogen on cancer have been reported and studies have been limited to different cancer types, and/or only in vitro cell models. It helps protect BABL/c mice from developing radiation-induced thymic lymphoma. Scientific studies give evidence that molecular hydrogen is the main reason behind improving the survival rate of mice-bearing colon carcinoma and inhibition of lung cancer progression. As a result of the findings, molecular hydrogen may have therapeutic benefit in the prevention and treatment of ovarian cancer.

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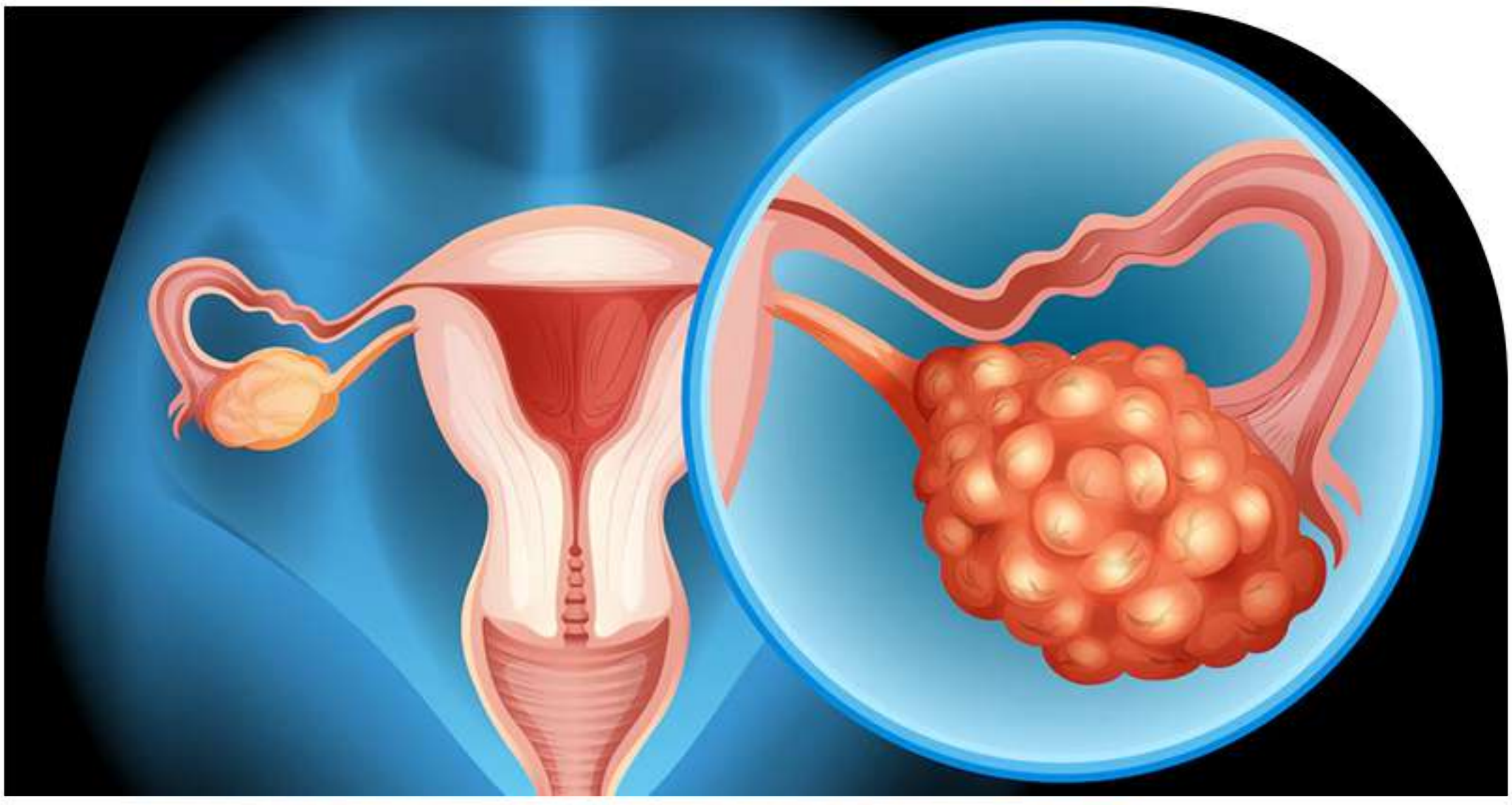
The goal of this study was to look into the potential therapeutic benefits of molecular hydrogen on ovarian cancer. Both in vivo and in vitro experimental models are used to assess molecular hydrogen's possible role. The underlying effects of molecular hydrogen on treating the medical condition of ovarian cancer were also explored.

Methods

• Animals and Experimental Design

Female athymic nude BALB/c mice bought from the Wei Tong Li Hua Company (Beijing, China) were kept in pathogen-free conditions. Studies were carried out with experimental protocols that involve animal study. These mice were kept in certain controlled conditions (25°C, 55% humidity and a 12 h day/night cycle) and provided standard laboratory food. For induction of subcutaneous ovarian tumor, serum-free culture medium was injected subcutaneously into their left flank.

14 days later, the mice with ovarian tumors were split into 2 groups: (I) mice in the control group (control, n=8) were conserved under normal conditions; (II) mice in the hydrogen treatment group (H, n=8) and were treated with H2 inhalation with a hydrogen-oxygen nebulizer machine for half an hour, 3 times daily.



The tumor growth was monitored with 2 bisecting diameters for each tumor with a caliper for 3 days during 6 weeks of undergoing hydrogen treatment. Its volume was calculated with this formula ($V = a \times b2/2$), where a represents the largest diameter and b the smallest diameter. When the 6-week treatment ended, mice were euthanized following their final 30 min H2 treatment. Tumors were completely removed, photographed, and fixed in 10% formalin/PBS or stored in liquid nitrogen for histologic examination.

• Hydrogen Treatment for Cell Culture

The ovarian cancer cell lines Hs38.T & PA-1 were kept in minimum essential medium with Earle's Balanced Salts with a 10% fetal bovine serum in a humid atmosphere of 5% CO2 at 37 °C. The cells were classified into two groups: (I) cells in the control group (control) were kept in complete medium; (II) cells in the hydrogen treatment group were placed in hydrogen-rich medium. The hydrogen concentration was monitored via a needle-type hydrogen sensor each week. The stick was replaced every 14 days for maintaining H2 concentration above 600 μM.

• Cell Viability Assay

Analysis was made using a cell counting kit-8 (CCK-8). Cells were kept in 96 well plates with a concentration of 5.0×103/well and cultured for 24 h. For growth curves development, cells were cultured for 0, 24, 48, 72, 96, and 120 h in hydrogen-rich medium.

• Cell Invasion Assay

The invasion ability of cells was determined with BD Matrigel Invasion Chambers based on the manufacturer's protocol. Top chambers with polycarbonate filters were coated with 50 μL of Matrigel. 1×105 cells in 100 μL serum free medium were kept to the top chamber & 650 μL hydrogen-rich medium was added at the bottom. Cells were allowed to migrate via the porous membrane at 37°C for 48 h. Cells present on the chamber's upper surface were removed with cotton swabs, while cells present on the chamber's lower surface were stained with 0.1% (w/v) crystal violet after fixing, 5 fields from each insert were calculated at 100× magnification.

• Cell Migration Assay

Cells were seeded in 6 well plates with a concentration of 1.0×106/well and kept for 24 h. plastic pipette tip helps draw a line across the cell surface in every single plate. The remaining cells were cleaned thrice with PBS for removal of floating cells and debris, followed by an incubation process for 48 hours in a hydrogen-rich medium. The images of the healing process were captured digitally at the time point of 0, 24 h after wounding. The healing assay for wound was performed in three independent experiments.

• Colony Formation Assay

1,000 cells per well were counted and placed in 6 well plates. These cells were incubated for 2 weeks in a hydrogen-rich medium and fixed with 4% paraformaldehyde. The cells are stained with 0.1% crystal violet. Independent triplicate experiments were performed and visible colonies were manually calculated.

• Sphere-Formation Assay

Cells were collected and washed for serum removal and suspended in serum-free DMEM or MEM/EBSS medium at a density of 2.0×103/mL. 500 cells per well were counted and placed onto ultra-low attachment 6 well plates for the sphere-formation assay. Cells were kept in a hydrogen-rich serum-free medium with B27 supplement, 20 ng/mL of epidermal growth factor (EGF), and 20 ng/mL of basic fibroblast growth factor (bFGF) (Pepro Tech).

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OUTCOME

Molecular Hydrogen Prevents Tumor Growth in Vivo

The molecular hydrogen effect on ovarian cancer biology in vivo, Hs38.T cells were xenografted onto nude mice and treated with hydrogen for about 42 days. The variation occurred in mean tumor volume and reached statistical significance at week 5. A marked reduction after hydrogen treatment was observed at weeks 5 and 6 when compared with the controls.

CONCLUSION

Molecular hydrogen exerts an anti-tumor role when related to ovarian cancer by suppressing the proliferation of CSC-like cells and angiogenesis.

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