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Anti-tumor Activity of DDP/DOX in Zebrafish

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Abstract

The tumor is one of the diseases that seriously endanger human health. In recent years, with the continuous exploration of model organisms, zebrafish has gradually been noticed and used by many researchers due to its many advantages. In the past anti-tumor basic research, mice mainly were used as experimental animals. Still, the breeding of mice involves many problems that zebrafish do not have, such as high cost, large breeding space, and high environmental requirements. Therefore, this study is based on the zebrafish model to explore the anti-tumor activity of cisplatin (DDP) and doxorubicin (DOX), which are broad-spectrum anticancer drugs in clinical practice, on zebrafish embryos. To provide new scientific ideas for the study of antitumor activity in zebrafish.

Keywords

Zebrafish; Tumor Xenografts; Cisplatin; Doxorubicin.

1. Introduction

Lung cancer, colorectal cancer, and breast cancer are the three most common malignant tumors in the world, of which colorectal cancer (intestinal cancer) is the second leading cause of cancer death, mostly concentrated in middle-aged and elderly individuals aged 40-60, while male The incidence of rectal cancer is higher than that of women [1]. This may be attributable to an aging population in developed regions, unfavorable modern dietary habits, and a gradual increase in risk factors such as smoking, physical inactivity, and obesity [2]. Tumors are a serious threat to human health, and in basic research, mice are often used as model organisms for anti-tumor research. But its economic cost has always been a big problem that many studies can't carry out [3].

Zebrafish belong to the Chordate phylum, Fish family, Cyprinidae teleost, and its genome is highly conserved, with more than 80% of homologous genes associated with human diseases [4,5]. Zebrafish embryos are basically formed within 48 hours after fertilization, including the heart, blood circulation, blood vessels, intestines, etc. Zebrafish are currently used for the study of organs such as the brain, eyes, blood, gut, blood vessels, endocrine, and heart [6,7].

2. Material

2.1 Animals and Cells

Wild-type zebrafish (6 to 12 months), GFP-labeled SW620 human colorectal cancer cell line.

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2.2 Reagents and Instruments

DMEM medium (Gibco), penicillin-streptomycin (Gibco), Pancreatin (Gibco), Fetal Bovine Serum (Gibco), Tricaine methane sulfonate (ULS), cisplatin (ULS), doxorubicin (ULS), sea salt (YanBao), Methyl Cellulose (Gentihold), culture plate, dish (Corning), Upright fluorescence microscope (Zeiss).

3. Experimental Methods

3.1 Tumor Cell Culture

Take the cell cryopreservation tube out of the liquid nitrogen tank and thaw it in a 37°C constant temperature water bath, so that the cells can be completely thawed within 3 minutes; quickly transfer the thawed cell suspension to the ultra-clean bench, and add complete medium. In the centrifuge tube, gently blow and beat evenly; transfer to a centrifuge at 1000 rpm for 5 min; remove the supernatant, flick the bottom of the centrifuge tube to disperse the precipitated cells, add 2 mL of complete medium, and blow gently, and then mix the cells The suspension was added to the T75 culture flask, the medium was supplemented to 15mL, shaken and put into the incubator, and the medium was changed to normal culture after 2 days.

3.2 Zebrafish Embryo Collection

Male and female zebrafish (1:1) aged 6 to 12 months were placed in the aquarium for fish breeding. After the plate was drawn and spawned the next day, the embryos were collected and put into a culture medium (0.2 g/L salt water) and cultured in a 28.5 °C incubator until fertilization for 48 hours. Each zebrafish underwent paired spawning only once a week. The rest of the feeding conditions were carried out in accordance with the conditions of the National Zebrafish Resource Center.

3.3 Tumor Cell Microinjection

Select 48 h fertilized AB line zebrafish embryos to remove the villi, and select normal developing embryos for injection; prepare tumor cells, resuspend the cells with 1 mL of DMEM after cell digestion and centrifugation, transfer 500 μL of the suspension to a 1.5 mL EP tube, and then tube with PBS Wash twice; remove the PBS supernatant, add 100 μL of DMEM to resuspend; count on the cell counter (107 orders of magnitude), after counting, re-blow the cells with a pipette tip and suck them into the capillary, and anesthetize the zebrafish with an anesthetic (0.32%). , the cells were injected into the yolk of the zebrafish, and the fish was returned to the incubator after washing with pure water to remove the anesthetic.

3.4 Drug Treatment

Twenty-four hours after injection, embryos with a similar tumor injection volume were selected under a fluorescence microscope and placed in a 96-well plate, and one zebrafish embryo was inserted into each well. After that, DDP/DOX was added to treat for 48 h. Then, the zebrafish were sucked out, washed with pure water, fixed on methylcellulose, and photographed to record the tumor size.

4. Results

4.1 Experimental Procedure

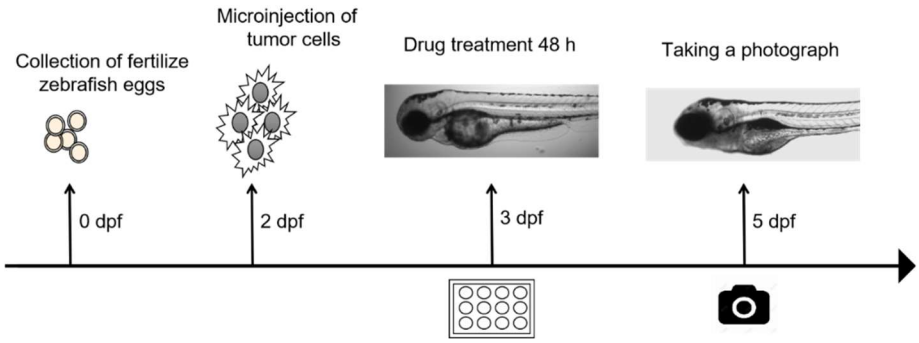

Figure 1. Flowchart (dpf means the time of fertilization)

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SW620 human colorectal cancer cells were used to detect the anti-tumor effect of DDP/DOX, and explore the schematic diagram of the experimental flow of zebrafish xenografts.

4.2 Growth of Tumor Cells in Zebrafish Embryos Could be Inhibited by DOX

The fertilized zebrafish embryos were collected and cultured to 48 hpf in a 28.5°C incubator. The main organs and tissues of the embryos at this stage had been developed. The SW620 tumor cells with green fluorescent protein were injected into the embryo yolk with a microinjector. On the second day, embryos with similar injection amount were selected and treated with DOX for 48 h. When the zebrafish grew to 5 dpf, the tumor changes were recorded by taking pictures under the microscope. The results are shown in the Figure 2, DOX can effectively inhibit the growth of tumor cells (no obvious toxic and side effects after drug treatment in zebrafish).

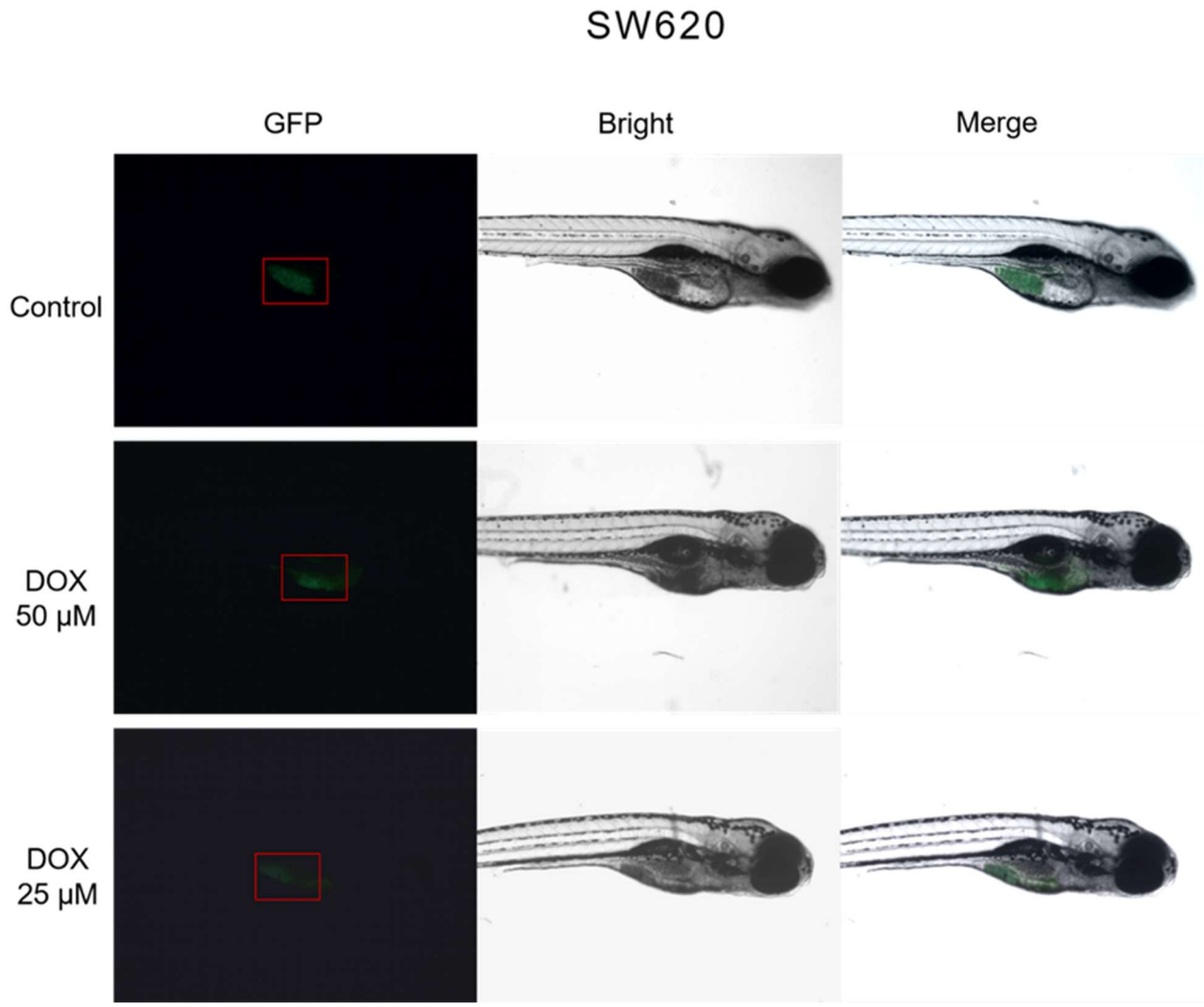

Figure 2. Antitumor activity of different concentrations of DOX in zebrafish.

4.3 Growth of Tumor Cells in Zebrafish Embryos Could be Inhibited by DDP

SW620 cells were injected into zebrafish embryos in the same way as above, and the embryos were cultured in a well plate and treated with DDP at different concentrations for 48 hours. The results are shown in the Figure 3: DDP at different concentrations can inhibit the tumor cells in zebrafish embryos.

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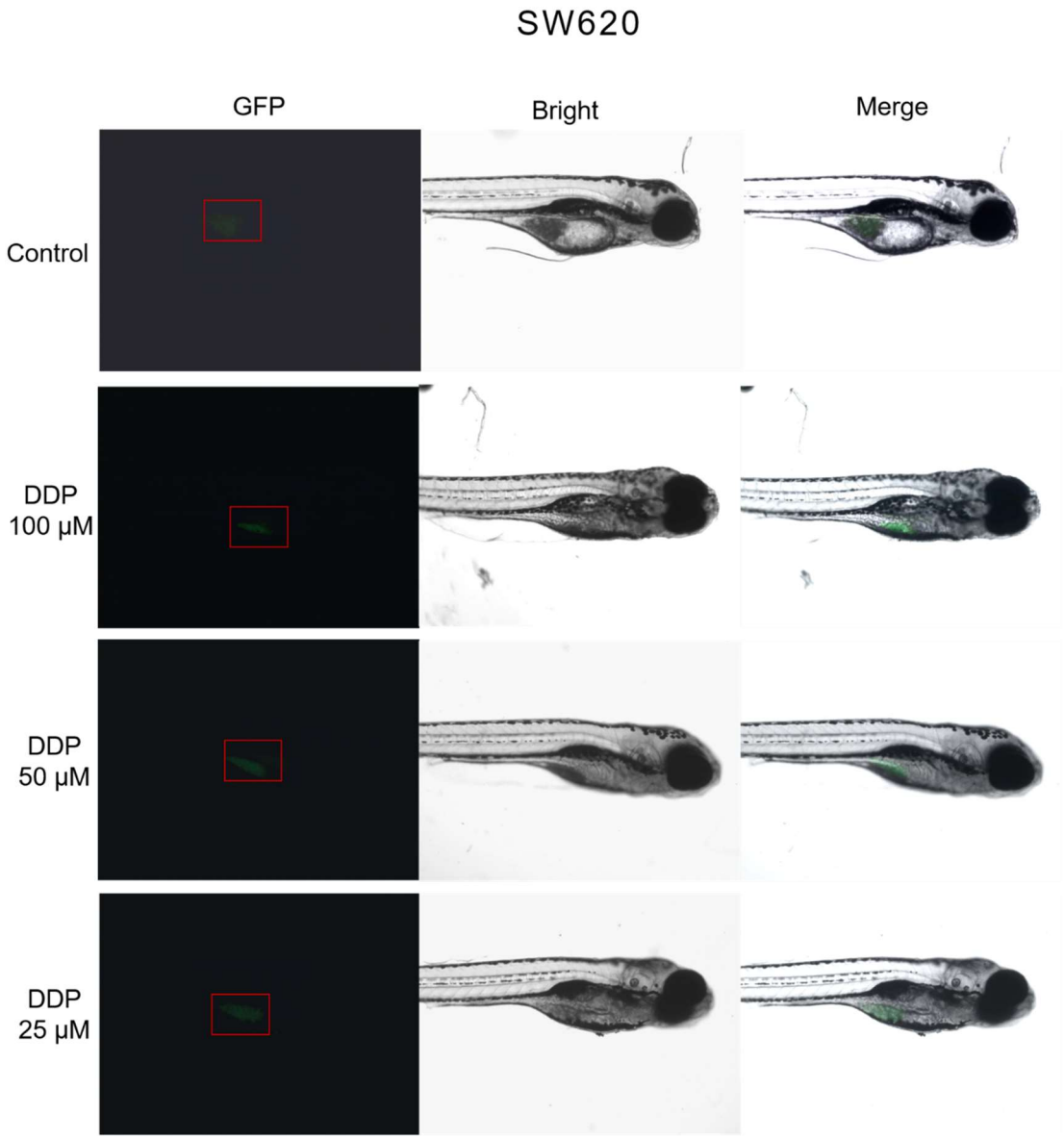

Figure 3. Antitumor activity of different concentrations of DDP in zebrafish.

5. Discussion

As a new model organism with high homology to humans, zebrafish in recent years have mostly focused on cardiovascular or neurological research in the direction of disease. It has the advantages of rapid development, a short reproductive cycle, early body transparency, and easy visualization. However, zebrafish also have inevitable defects. Given its rapid growth, it is not suitable for the study of chronic diseases such as heart failure. For the research on the antitumor activity of drugs, the common xenograft model studies are mostly carried out in mice. This kind of model takes a long time to build, the cost is high, and the operation is more difficult than zebrafish. Therefore, in this study, the antitumor activity of commonly used broad-spectrum anticancer drugs in zebrafish was studied. To study the effects of DDP and DOX on the growth of SW620 human colorectal cancer cells in zebrafish. The experimental results show that both can effectively inhibit the growth of tumor cells in zebrafish within the non-toxic concentration range. This study provides a scientific basis for zebrafish as a tumor xenograft model. Cisplatin and doxorubicin, as clinical anticancer drugs, have certain drug toxicity and they could inhibit the growth of tumors in zebrafish within the non-toxic range. This study can provide scientific ideas for subsequent antitumor drug activity research.

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