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Zebrafish Bronchial Cancer Model

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Abstract

Cytoskeleton Regulator RNA (CYTOR) is essential for the growth of human cancer. In comparison to mouse xenograft technology, zebrafish xenograft approach is more frequently used in human cancer research. This model facilitates the study of CYTOR RNA activity *in vivo*. It is advantageous to comprehend how CYTOR RNA works in the growth and invasion of lung cancer cells. Zebrafish xenografts provide evidence for the *in vivo* role of CYTOR in research. *In vivo* confocal imaging helped pinpoint the specific roles of CYTOR in cell proliferation. Additionally, research on zebrafish xenograft models and cultured cells were done to see if CYTOR might be used medicinally to treat tumors. CYTOR In SPCA1 and in the A549 lung cancer cell line, CYTOR inhibition was shown to diminish cell proliferation and invasion in conjunction with an EGFR inhibitor in utero. Human alveolar basal epithelial cells that is cyanogenic. Knockdown of CYTOR reduced lung cancer cell proliferation, growth, and migration in a zebrafish xenograft model, as observed by two imaging techniques at different magnifications. These cells, also known as A549 cells, were the first isolated and cultured from malignant lung tissue found in a tumor discovered in a 58-year-old Caucasian man in 1972. Additionally, afatinib's capacity to slow the progression of lung cancer in zebrafish xenograft models and cultured lung cancer cells was enhanced by the suppression of CYTOR. Our study illustrates the oncogenic activities and tumor treatment potential of CYTOR in lung cancer using a zebrafish xenograft model. As a result, this model can be applied to functional and application studies of the RNAs that regulate the human cytoskeleton in the biology of tumors.

Introduction

Zebrafish (Danio rerio), a small freshwater fish, are used in biomedical research as model organisms. George Streisinger (1927-1984) was a pioneer in the field of zebrafish research. Zebrafish xenografts have become more widely used recently as an animal model for research on human cancer during the past ten years. Zebrafish xenografts of numerous cancers, such as melanoma, colorectal cancer, breast cancer, etc., have so far been created. Comparatively to the mouse xenografts model, the transparent zebrafish larval xenografts model offers the speed and intuitiveness for investigations on the cellular resolution level with reasonably large samples, which would add to the knowledge of cancer biology. Developmental biology has been the main focus of zebrafish study; maintaining and watching zebrafish in the lab resulted in the discovery of illnesses similar to cancer that subsequently became a research issue. As a consequence, since 2000, around 50 researches that employed zebrafish as a cancer model have been published. Only a few of the techniques used include carcinogenic therapies, transplanting mammalian cancer cells, forward genetic screening for genomic instability of proliferation, reverse genetic target selected mutagenesis to inactivate known tumor suppressor genes, and the development of transgenic to express human oncogenes. Zebrafish has been shown to develop practically every type of human cancer with a comparable shape and equivalent among diverse mutations, and they often manifest in later life, according to gene expression array analyses. On occasion, variations between cancer spectrums in mice and humans are seen. However, the zebrafish model has made a name for itself in cancer research by augmenting existing models with its own experimental benefits and characteristics. Examples of these include delivering chemical substances, tracking the growth of tumors in actual fish using fluorescence imaging, and investigating the use of genetic enhancers and suppressors in addition to chemical modifiers. This paper aims to provide a comprehensive overview of zebrafish research as a model for bronchial cancer. There are a few instances where the tumor spectrum in mice and humans diverge. However, the zebrafish model has made a name for itself in cancer research by augmenting existing models with its own experimental benefits and characteristics. Examples of these include delivering chemical substances, tracking the growth of tumors in actual fish using fluorescence imaging, and investigating the use of genetic enhancers and suppressors in addition to chemical modifiers. This paper aims to provide a comprehensive overview of zebrafish research as a model for bronchial cancer [1-4].

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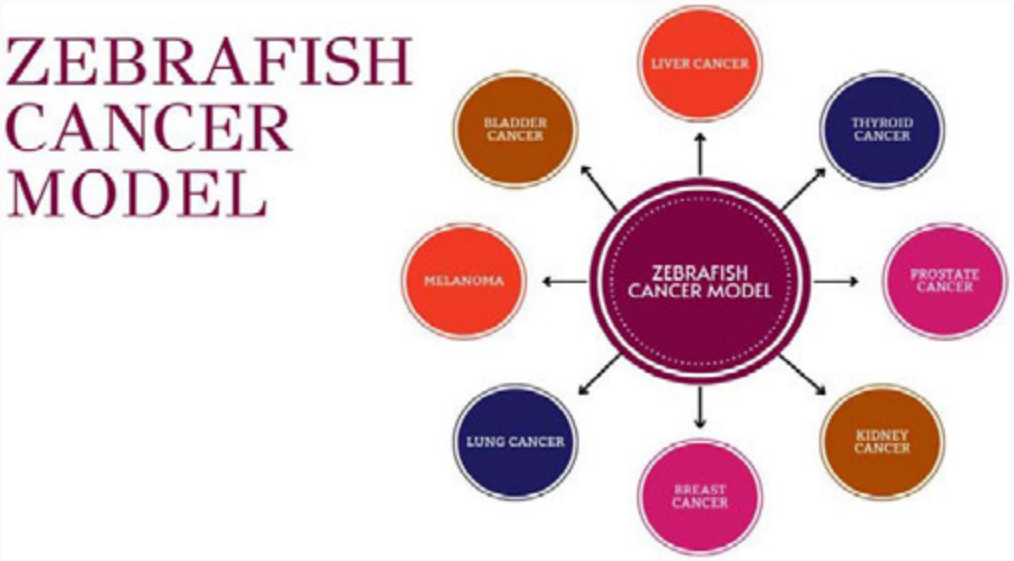

Figure 1: Zebrafish cancer models.

Reason

The biggest risk factor for lung cancer is smoking, but there are many less well-known risk factors include the environment and genetics. Lung cancer has been linked to environmental exposure to radon, asbestos, arsenic, beryllium, and uranium. Additionally, factors that raise the risk of lung cancer include age, family history, radiation to the chest area, and lung conditions including chronic obstructive pulmonary disease (9COPD).

The second most prevalent type of cancer in the world, lung cancer is the main reason for cancer-related fatalities in both men and women (Figure 1).

Preparing Tumor Cell from Cell Culture Zebrafish

The average temperature for adult zebra fish is 26°C to 28.5°C with a 14-h light/dark cycle. However, the 10% Hank's solution, which contains 140 NaCl, 5.4 KCL, 0.25 Na_2HPO_4, 0.44 KH_2PO_4, 1.3 $CaCl_2$, 1.0 $Mgso_4$, and 4.2 $Na|HCO_3$, brought the temperature to the ideal level for embryonic growth. In our research, zebrafish AB natural type and transgenic line Tg were both employed. The human bronchial epithelial cells used to make the human non-small lung cancer cell lines A549, H1299, H1975, SPCA1, Pc9, and 16NBE were taken from a 1-year-old heart-lung patient immortalized with SV40 plasmid. In 1640 media, A549, H1975, and 16HBE cells were cultured, whereas /h1299, SPCA1, and PC9 cells were grown in DMEM. FBS and 100 u/ml of streptomycin were added to both media, and the cultures were carried out at 37°C in a humidified air environment containing 5% CO_2. The most used technique for removing total RNA from cell lines is the Trizol procedure [5]. Using a first-strand cDNA synthesis supremix for qPCR kit, a CDNA product that has been directly synthesized from whole RNA. To track the expression level of LINC00152 in several lung cancer cell lines, a real-time PCR experiment was conducted using a SYBR green master mix kit in accordance with the manufacturer's instructions. Data were assessed using comparisons. Different lung cancer cell lines have LINC00152. Data were assessed using comparisons. General Bio Systems (CNINa) sold us LINC00152 small interfering RNA (siRNA) and negative control siRNA [6]. After 24 h, particular siRNAs were transfected into A549 and SPCa1 cell lines using Lipofectamine 3000 reagent (Invitrogen, USA). The A549 and SOCA1 cell lines were grown in six-well plates. The siRNA sequence for LINC00152 is 5'- TGATCGAATATGACAGACACCGAAA-3', whereas the siRNA sequence for the negative control is 5'-TTCTCCGAACGTGTCACGT-3'. After transfection for 24 h =, cells were collected, and knockdown effectiveness was then measured by qRT-PCR. Cell viability can be determined using sensitive colorimetric tests with the Cell Counting Kit (CCK-8). A549 and SPCA1 cell lines were transfected with LINC00152 SiRNA and a negative control SiRNA before being seeded in 96-well plates after 24 h. In 96-well plates, 2000 cells were planted in each well. According to the manufacturer's instructions, the optical density at 450 nm was measured using a microplate reader (bioTek ELX800, USA) every 24 h from 0 to 72 h to monitor cell growth. The data from each group were then examined. The LINC00152 SiRNA and a negative control SiRNA were transfected into the A549 and SPCA1 cell lines. The cells were transfected for 24 h before being planted onto 24-well plates with 8 mm -pored chamber crickets. Between 40000 and 50000 cells were plated in the upper chambers after they had been diluted with serum-free media. The lower chamber of 4-well plates containing 800 ul of medium containing 10% FBS was then filled with the upper chamber. Cells were moved to the membrane's base surface after 24 h, mixed with methanol, and stained for 30 min with 0.1% crystal violet. Images were captured using an upside-down microscope. Cancer cells were marked with CM-Dil (Invitrogen, USA) before to injection. The cells were collected, collected three times, and then washed with HBBS. After that, the cells were stained with CM-Dil for five minutes at 37°C. Incorporated dye was then removed by washing three times in HBBS after 15 min at 4°C. The cells were then analyzed using fluorescence microscopy. Zebrafish larvae were then mounted using 1.2% low-melting (pvs) of the subsequent injection. At 24 h post-injection (hpi), the zebrafish larvae with transplanted cells of identical sizes were removed for additional analysis, and they were subsequently grown at 34°C until the end of the research. For the imaging tests, the zebrafish larvae were mounted with 1.2% low-melting gel four days after injection (bpi). Using stereomicroscopy

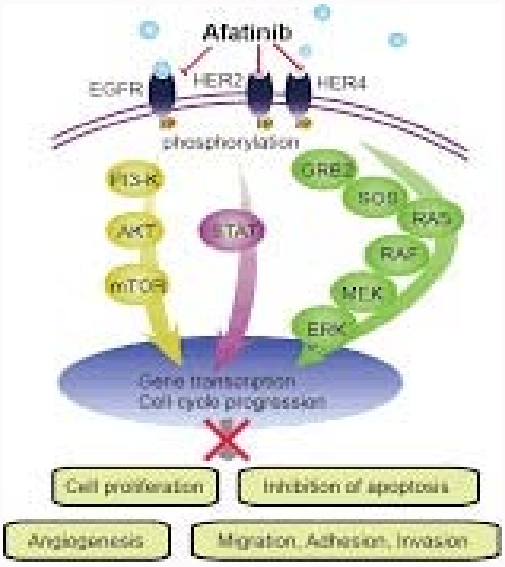

Figure 2: Afatininb process.

(MVX10, Olympics, and Japan) or a confocal magnifier with a 20x water-drenching objective (Fluoview 1000) or 1600x1200 pixels (MVX10), imaging experiments were conducted.

Drug Administration

After the cells were sown in 96-well plates and cultivated for 24 h in media with 2 nm afatinib (Seleck, China), cell proliferation was assessed by ccl-8. Transfected cells were also plated into the upper chambers, and 2 nm afatinib was present in the medium of the bottom chambers. The cell incursion was then assessed. The zebrafish xenograft at 24 h post-implantation was randomly split into two groups, one of Hank's solution and the other of Hank's solution with 1 nM afatinib added. These larvae were mounted for the imaging tests after being raised for three days [7].

Result

The possibility of LINC00152 as a lung cancer therapeutic target was then evaluated. One important therapeutic target for NSCLC lung cancer and the cell lines is EGFR. A549 also demonstrated its sensitivity to different EGFR inhibitors, and LINC00152 was announced as a knockdown agent that can lower EGFR expression. Based on this, it may be concluded that LINC00152 can control the EGFR pathway, which controls the development of cancer. First, we looked at viability following LINC00152 knockdown. It has an earlier inhibitory impact than A549 indicating that the EGFR pathway may be upstream of LINC00152. Concurrently stopping LINC00152 and afatinib in addicts may improve the inhibitory effect. The transwell assay in A549 cells similarly showed inhibition of penetration. These findings imply that EGFR inhibition and LINC00152 silencing may have an additive effect on lung cell development (Figure 2).

Conclusion

In conclusion, our research showed that LINC00152 causes cancer *in vivo* utilizing zebrafish xenografts. Pharmacological tests also had shown a synergistic impact between inhibiting EGFR and reducing LINC00152 in halting the spread of lung cancer. Our investigation suggests that zebrafish xenograft can be used in IncRN study.

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