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Zebrafish Model in Cancer Research

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Abstract:

The zebrafish (*Danio rerio*) is a highly effective non-mammalian vertebrate model that has been extensively used to study development and disease, most recently cancer. The significant evolutionary conservation of cancer-related programmes across humans and zebrafish facilitate the extrapolation of fish-to-human studies. These advantages include minimal maintenance costs, dynamic monitoring of tumour progression in vivo, and the capacity to screen compounds in large numbers of animals at a low cost. Novel tumour growth models, such as electroporation of transgenes into adult zebrafish, may help us better understand the spatial and temporal control of cancer genesis and progression *in vivo*. Examining both genetic and epigenetic changes may be crucial for unravelling the pathophysiology of such a complex disease like cancer. Oncology researchers have recently recognised the viability of using zebrafish to examine human cancers and their potential to determine the invasiveness of patient-derived xenograft cells. Recent advances in zebrafish xenotransplantation research and drug screening have demonstrated that zebrafish is a reliable model for studying human cancer and may be useful for assessing the invasiveness of patient-derived xenograft cells. A rapid, large-scale study of *in vivo* drug reactions and kinetics in zebrafish has the potential to revolutionise personalised medicine and combination therapy. Thus, together with the mouse, zebrafish is rapidly approaching a future as a pre-clinical cancer model.

Keywords **—Antiproliferation, Anti-angiogenesis, cancer microenvironment, zebrafish**

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I. INTRODUCTION

The zebrafish (*Danio rerio*) have surfaced to the world of cancer as they became more well known(1, 2). With the early studies in 1930, using the zebrafish for studying and developing different cancers has been more common and been very promising (1, 3). The high level of similarity between humans and zebrafish genetics and homology such as the brain and immune system etc. offers unique opportunities for scientists to study and conduct more experiments on cancer which could never be done on other animals. It has been found that zebrafish contain more than 70% of human genes (4). The flexibility and adaptability of the zebrafish also allow scientists to freely manipulate its genetics (5). Zebrafish bodies are made up of a brain, intestine, mouth, spinal cord, liver, pancreas, bile ducts, kidney, esophagus, heart, muscle, nose, blood bone, and cartilage (6, 7). These include many important genes and pathways that both organisms contain. Hypothetical diseases that happen to human body parts could be modeled if zebrafish also contain the specified body part (1).

One advantage of using zebrafish is the high reproductive rate. Zebrafish are able to provide scientists with more test subjects approximately every ten days, zebrafish can produce 50 to as many as 300 eggs (8). Experiments normally take more than one trial as they are often repeated in order to get the most accurate result. The more the trial conducted the more accurate the result is (9). Additionally, most major organs of the zebrafish have completely developed within 24 hours (9).

While zebrafish embryos are laid and fertilized externally, mice embryos develop internally inside their mother (9). The internal fertilization of mice embryos makes it hard for scientists to access and manipulate genetic makeup, unlike zebrafish. Zebrafish embryos that are fertilized externally can be easily injected with DNA or RNA to manipulate their genetics at the early stage (10). In order to manipulate mouse embryos, the mother needs to be sacrificed (10, 11). Afterward, the mouse embryos also needed to be transplanted into another female mouse to keep alive. Working with mouse embryos can be complicated at certain times and can be easily avoided if zebrafish were used instead (12, 13). The transparent embryos of zebrafish are one of the most important aspects. Mouse embryos are not clear and are unable to see through because it was developed inside its mother (14). However, for zebrafish, scientists were able to observe the fertilized eggs slowly growing into baby fish under a microscope (15, 16). The transparent embryos of the zebrafish also allow tumor metastasis, purification, and angiogenesis to be modeled and visualize at a cellular molecular level (17, 18). The purpose of this review is to focus on the advantages and disadvantages of using zebrafish xenotransplantation in cancer research on proliferation, angiogenesis, metastasis, and tumor microenvironment. As zebrafish models are unique and easy to manipulate which can be a great platform for advanced cancer research in the future.

II. ANTIPROLIFERATION

One option to inhibit proliferation would be to increase the apoptosis of cells or the death of it to keep it controllable (19). However, it has been shown that some tumors have the ability to evade apoptosis which means the cancer cell refuses to die and leads to proliferation (20, 21). This leads to the second option which is to decrease the rate of replication of cells or anti-proliferation (22, 23). In order to see proliferation clearly and figure out the solution, the cancer cells were stained with a fluorescent dye, allowing them to observe the growth of cancer tumors in real-time under fluorescent microscopy (24-26). In order to investigate the antiproliferative activity of any substances, observing the expression of antiproliferative markers will be determined (20).

On the report from Siew Hong Lam &Zhiyuan Gong, cyclin genes (ccnd1, ccnd1, and ccnt), pcna, pold1, pold3, cdk2, cdk8, sch1, mapk1, chc1, grn, pa2g4a, rpa1, anapc4, and cseil indicate a high activity of proliferation meaning it upregulated DNA replication and tumor samples. However, anti-apoptotic signal (tegt) have been downregulated due to genes that are related to cell apoptosis which include tp53, rbbp4, lgals1, eif4g2a, bax, hdac1, apaf1, and rad21 (27). Wnt beta Catenin pathway in zebrafish, also associated with human liver cancers, was also observed. The observation suggested the deregulation of pathways die the deregulation of ctnnb, wif1, wnt2, ctnnbip1, and ccnd1 (27). The upregulation of shc1, mapk1, dusp4, dusp6, and genes associated with Ras GTPases (e.g., rhoc, rhogap1, cdc42, rac1, g3bp2 and gef10) have shown to be affected by the activation of Ras-MAPK pathway in zebrafish (27, 28). The disturbance of embryonic hematopoiesis, including expansion of the myeloid compartment and anemia, in embryos was the result of spi1 promoter which was expressing tel-jak2a oncogene (29). Human liver neoplasia and zebrafish have shown many molecular similarities from comparative microarray analyses. Common molecular signature of human liver cancer and some molecular resembles extending to the development of liver tumors have been spotted in zebrafish liver tumors, suggesting an even higher level of similarities between the two (30, 31). The clinical potential as distinguishing indicator and/or curative targets of the genes in liver neoplasia have an emphasis on the complementary of liver tumors in humans and fish (32, 33). The use of the zebrafish model in colorectalcarcinoma has been performed byusing the zebrafish-HCT-116 xenograft model the activity of complexes 1 and 4 against human colorectal carcinoma *in vivo* was investigated (34, 35). At an early stage, the yolk of Tg(fli1:EGFP) embryos are fluorescently labeled and injected with HCT-116 cells (36). Three days after the injection, using fluorescence microscopy, zebrafish xenografts were evaluated to observe the effect of tumor mass development that applied

complexes, tumor neo-angiogenesis, and cancer metastasis and dissemination (36).

II. ANTI-ANGIOGENESIS

Nicoli et al. (2007) developed the VEGFR2:G-RCFP zebrafish using transgenic zebrafish embryos that reveal green fluorescent protein at their vessels. SU5402- and SU5416-treated anti-angiogenesis were conducted (37). The results showed that repress tumor stimulates angiogenesis by analyzing SIVs in zebrafish embryos. The zebrafish tumour xenograft model provides an innovative new cancer model system (38). The process of tumour growth and metastasis is closely related to angiogenesis. The teleost zebrafish is a promising platform in cancer investigation . The observed facts appear to lend further credence to these ideas, as in vitro rFGF2 stimulation activates signaling in fli1:EGFP cells separated from transgenic tg(fli1:EGFP) y1 zebrafish embryos. When co-injected with growth factor or mixed with fish water, SU5402 and long-pentraxin 3 preven the agiogenic activity of rFGF2. Additionally, identical to rFGF2, the zebrafish VEGF-A injection promotes a noticeable angiogenic feedback in the ZFYM assay, which the VEGF receptor-2/KDR TK inhibitor SU5416 was the one that suppressed (39, 40). The ZFYM assay is a novel method to screen for inhibitors of a particular angiogenic growth factor in zebrafish. The assay offers notable benefits over alternative animal models (41).

Zhang J et al., 2018 studies on the mechanism of bevacizumab's antiangiogenesis activity were predominantly conducted on mouse models, and thus there are no visual or intuitive models for observing the antiangiogenesis process (42). They first investigated bevacizumab's ability to inhibit angiogenesis using a zebrafish model (42). Bevacizumab prevents zebrafish from developing subintestinal veins, a process that mimics tumour angiogenesis *in vivo* (42). At the same time, bevacizumab impairs the shaping of particular vasculature in subintestinal veins but not in the trunk. Additionally, this study demonstrated that zebrafish retinal angiogenesis can be greatly inhibited by bevacizumab, which may have implications for treatment (42). Experimental evidence has shown that zebrafish embryonic development may serve as a target for anti-angiogenic compounds (43, 44). Zebrafish offer distinct advantages for drug discovery about anti-angiogenic potential, including their high genetic, physiological, and pharmacological resemblance to humans. The inherent in vivo imaging capabilities of zebrafish embryos have been enhanced through transgenic technology (45, 46). This allows vessels and blood flow to be observed and recorded in real-time, for example, using transgenic Tg(fli1a: EGFP)y1 zebrafish embryos, in which ECs express the EGFP under the control of the fli1 promoter, which allows for live monitoring and blood vessels time-lapse recording (47).

III. ANTIMETASTASIS

Several studies have indicated that the close monitoring of individual cells and organismal structures in real time is possible due to the optical clarity benefit of using zebrafish to examine metastasis events (36, 48-51). Not only that but, the combination of genetic manipulation tools and fluorescence also made it possible for each step of a malignant cell's journey such as the invasion and the colonization to be interrogated *in vivo*(52, 53). According to Teng et al, not only was the establishment of the zebrafish-metastasis model successful but, the inherent metastatic phenotypes of human cancer cells, genetic regulation of tumor metastasis was also illustrated and preserved in zebrafish (48).

IV. CANCER MICROENVIRONMENT

The tumour microenvironment (TME) is a complex and dynamic environment in that tumours have strategically evolved to aid in their survival and dissemination. In recent years, the focus has been on characterizing and identifying the tumour-supporting roles and the potential of the TME components to be targeted (54). However, it has been intrinsically challenging to recapitulate the human TME and leave a lot to be investigated. In this respect, in vivo models such as zebrafish have proved essential for TME modeling and investigation, with their optical clarity, convenient genetic manipulation and high level of engraftment

(8, 54). Fully grown zebrafish larvae and immunocompromised adults readily engraft human cancer cells, which have been shown to have comparable growth kinetics and histology to those grown in mice (55). Additionally, they demonstrated that zebrafish tumour xenografts could be used to accurately assess the metastatic potential of human cancer cell lines and primary tumours, allowing for the robust assessment of single-gene mutations (48, 56, 57). Considering that it is very inexpensive, has a very rapid speed, and possesses impressive throughput capacity, the zebrafish has demonstrated that it is a well-positioned research model for fundamental studies and is getting ready to join the arena of personalized medicine.

CONCLUSIONS

Zebrafish is a reliable model for simulating and visualizing the biology and dynamics of human cancer cells in vivo, including metastases and tumour tissue neo-angiogenesis. Additionally, the role of epigenetic modulators in tumour biology may help us better understand such complex diseases as cancer. The availability of transgenic and mutant models and the ability to transplant cancer cells into zebrafish opens up a world of possibilities for human cancer to be investigate further. Although zebrafish is not a mammalian model organism, it shares striking evolutionary similarities with humans in disease-related genes and pathways. While large-scale in vitro drug discovery is possible, the effect on the entire living organism may be significantly different. Screening for targeted therapy in zebrafish xenografts may open up new avenues for personalized anticancer therapy in the future, as new research has demonstrated that zebrafish models of human cancer are reliable. In summary, zebrafish are a valuable resource for cancer researchers because they provide several unique benefits for studying the mechanisms underlying cancer formation and progression. Optically transparent, genetically tractable, and amenable to pharmacological testing are at the top of the list. Unsurprisingly, cancer researchers are paying attention, as these characteristics open up new avenues for significant discoveries and provide a cost-effective whole animal model for therapeutic development. Now that zebrafish have gained the attention of cancer researchers, it will be fascinating to watch the progression of zebrafish cancer research over the next few years as it fulfils its numerous promises.

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