Academy of Romanian Scientists
Annals Series on Biological Sciences

Volume 13, No. 2, 2024, pp. 101 - 108
Online ISSN 2285 – 4177
ORIGINAL PAPERS

CARCINOGENIC POTENTIAL OF CERTAIN CHEMICAL AGENTS IN ZEBRAFISH: A SHORT REVIEW

Cătălina IONESCU[1,2*], Viorica RARINCA[2,3,4], Mălina VISTERNICU[1,2], Alin CIOBICA[1,2,5,6], Fatima Zahra KAMAL[7,8]

[1] Department of Biology, Faculty of Biology, Alexandru Ioan Cuza University of Iasi, Bd. Carol I no. 20A, 700505 Iasi, Romania.
[2] "Ioan Haulica" Institute, Apollonia University, Pacurari Street 11, 700511 Iasi, Romania
[3] Doctoral School of Biology, Faculty of Biology, Alexandru Ioan Cuza University of Iasi, No 20A, Carol I Avenue, 700506 Iasi, Romania
[4] Doctoral School of Geosciences, Faculty of Geography and Geology, "Alexandru Ioan Cuza" University of Iași, Carol I Avenue, No. 20A, 700505 Iași, Romania
[5] Center of Biomedical Research, Romanian Academy, No 8, Carol I Avenue, 700506 Iasi, Romania
[6] Academy of Romanian Scientists
[7] Higher Institute of Nursing Professions and Health Technical (ISPITS), Marrakech, Morocco;
[8] Laboratory of Physical Chemistry of Processes and Materials, Faculty of Sciences and Techniques, Hassan First University, B.P. 539, Settat 26000, Morocco.
[*] Corresponding author: catalinaionescu81@yahoo.com

Abstract. *Zebrafish are an increasingly valuable model for cancer research due to their rapid development, genetic similarities to humans, and optical transparency, which allows real-time tumor observation. These fish have been used to study various cancers through exposure to carcinogens. Additionally, zebrafish share key genetic traits with humans, making them ideal for studying tumor mechanisms and testing therapies. Their ability to model carcinogenesis, track tumor progression non-invasively, and evaluate environmental toxins highlights their potential in advancing cancer research and drug discovery.*

Keywords: *Zebrafish*, Carcinogenic, Animal mode, Cancer Tumor Development

DOI [10.56082/annalsarscibio.2024.2.101](https://doi.org/10.56082/annalsarscibio.2024.2.101)

1. *Zebrafish* – What Makes this Animal Model Reliable for Cancer Research?

Fish have long been used as valuable models for studying cancer, offering several key advantages for biomedical research, which are often unavailable in other animal models [1]. One of the earliest cancer models in animals was developed using platyfish (*Xiphophorus*). Researchers discovered that genetic hybrids between pigmented platyfish (*Xiphophorus maculatus*) and non-pigmented swordtails (*Xiphophorus helleri*) naturally develop melanoma. This model is one of the earliest and most important in cancer research. Additionally, medaka (*Oryzias latipes*), a small freshwater fish, has provided valuable insights into the study of cancer, especially in understanding melanoma pathogenesis [2].

Zebrafish (*Danio rerio*) are well-suited for tumor modeling through various methods, such as chemical treatments, genetic knockouts, gene overexpression, and xenotransplantation. They have been utilized to study a wide range of cancers, including skin, pancreatic, breast, and lung cancers, as well as leukemia and glioma [3].

A key feature of this model is the optical transparency of embryos and larvae, which facilitates real-time visualization of tissue morphogenesis, particularly when combined with transgenic reporter lines. This capability is critical for studying gene function and addressing behavioral research questions [4]. The popularity of zebrafish as a research model is also driven by their rapid ex utero development and reproductive efficiency, with females capable of producing 200–300 eggs every 5–7 days. Organ systems such as the digestive, nervous, and cardiovascular systems develop within one week, enabling accelerated studies of physiological and pathological mechanisms through live-cell imaging [3]. Furthermore, zebrafish share significant genetic and physiological similarities with humans, approximately 70% gene similarity, including a comparable innate immune system, making them particularly relevant for cancer research. Additional advantages of zebrafish include cost-efficiency, small size, and ease of maintenance in minimal space. Their embryos are highly accessible for manipulation, and they can be studied at any developmental stage, from larvae (3–29 days post-fertilization) to adults (90 days to 2 years). These attributes, along with their rapid development, optical transparency, and genetic tractability, position zebrafish as an indispensable model for biological research and drug discovery, particularly in oncology and nanomedicine [5].

2. *Zebrafish* as a Model for Chemical Carcinogenesis and Tumor Development

Zebrafish have been observed to develop various diseases, including cancer, under laboratory conditions. Research has demonstrated that zebrafish can spontaneously develop a wide range of tumors, with the most frequently affected tissues being the testis, gut, thyroid, liver, peripheral nerves, connective tissue, and ultimobranchial gland. Less commonly, tumors have been found in blood vessels, brain, gills, nasal epithelium, and the lymphomyeloid system [6]. This makes zebrafish a valuable model for studying carcinogenesis, a multi-step process characterized by molecular and cellular alterations that progress through initiation, promotion, and progression stages [7].

Carcinogenesis in zebrafish is induced by a combination of genetic mutations and environmental factors, which disrupt the balance between oncogenes and tumor suppressor genes. These genetic and epigenetic changes enable cancer cells to proliferate excessively, evade immune surveillance, and metastasize [8]. *Zebrafish* have proven particularly useful in chemical carcinogenesis studies, where small molecules are used to perturb biological pathways. This approach not only provides insights into developmental and disease mechanisms but also serves as a platform for discovering new cancer therapies [9].

Fish species have been extensively used to study the effects of various chemical carcinogens, helping to evaluate their effectiveness in fish, model human carcinogen exposure, and assess environmental toxins. The most common methods of exposing zebrafish to carcinogens are through water and diet. Other approaches used in fish models include embryo injection and topical application. In comparison, rodent models are typically exposed to carcinogens through feed or water, gastric lavage, inhalation, injection, or topical application. While the routes of exposure may differ between fish and mammals in carcinogenicity studies, the liver remains a primary target organ for many carcinogens in both species [10].

The first studies on chemical carcinogenesis in *zebrafish* began in the 1960s, when Stanton demonstrated that exposing zebrafish fry to diethylnitrosamine induced liver neoplasms similar to those seen in rodents [11]. Subsequent research expanded the repertoire of carcinogens used, including ethylnitrosourea (ENU), 7,12-dimethylbenz[a]anthracene (DMBA), and N-methyl-nitrosoguanidine (MNNG), which induce various cancers, such as skin papillomas, hepatic adenomas, and rhabdomyosarcoma [12]. Spitsbergen and colleagues conducted comprehensive studies showing that while zebrafish rarely develop spontaneous tumors, exposure to carcinogens reliably induces neoplasms across many tissues, depending on the carcinogen, the fish's age, and genetic strain [13,14].

Zebrafish offer numerous advantages for cancer research, including the ability to create models using reverse and forward genetics, as well as chemical and genetic screening. The use of physiologically intact vertebrates for small-molecule screening adds another dimension to studying cancer biology and identifying potential drug targets [15]. Moreover, their established role in chemical carcinogenesis and advancements in zebrafish genomics have significantly contributed to understanding liver carcinogenesis and other cancer types [16].

3. Chemical Agents with Carcinogenic Potential

Zebrafish have become a valuable model in carcinogenesis research due to the ease with which they can be exposed to carcinogenic compounds. These chemicals can be effectively dissolved in water or added to their diet, allowing for prolonged exposure, which is essential for studying long-term tumor development. Early studies focused on liver and intestinal cancers in zebrafish and other species like guppies, following exposure to compounds such as DMBA, N-nitrosodimethylamine (NDMA), and N-nitrosodiethylamine (NDEA). More recent research has expanded on these findings, confirming that NDEA predominantly induces liver and pancreatic carcinomas, while NDMA causes only liver tumors. DMBA, however, induces a wide variety of tumors across multiple organs, including the liver, intestines, pancreas, thyroid, testes, and even mesenchymal tissues like cartilage, blood vessels, and muscles [6].

Further studies have explored the diverse types of tumors induced by different carcinogenic compounds in zebrafish. For instance, MNNG, a potent carcinogen, leads to tumor formation in blood vessels and testes, such as hemangiomas and seminomas, when larvae are exposed for extended periods. Similarly, exposure to DMBA has been shown to result in liver neoplasms, along with other forms of cancer, including chordomas and chondrosarcomas. Additionally, ENU, commonly used for mutagenesis screens, also causes skin papillomas but does not lead to invasive skin cancers. Other carcinogens, such as NDMA, have been tested in zebrafish, where exposure typically results in liver and bile duct tumors, including hepatocellular carcinomas and cholangiocarcinomas [17].

Zebrafish have also been valuable in studying cancer susceptibility in genetically predisposed mutants. Studies have shown that mutant zebrafish, which have a lower spontaneous tumor incidence, exhibit increased sensitivity to carcinogens compared to wild-type zebrafish. This makes the zebrafish model particularly useful for evaluating how genetic factors influence cancer progression. For example, exposing vhl+/− *zebrafish* to DMBA led to an increase in liver, bile duct, and intestinal tumors, demonstrating how specific carcinogens can trigger organ-specific tumorigenesis [18].

These studies highlight the versatility of zebrafish as a model for cancer research, showing their capacity to develop a wide range of tumors across multiple organs when exposed to various carcinogens. This makes *zebrafish* an invaluable tool for understanding cancer mechanisms and testing potential anti-cancer therapies, as the tumor types induced often mirror those found in human cancers.

Furthermore, several carcinogenic substances have been shown to induce human-like tumors in zebrafish, making them even more valuable in cancer research. Research into MNNG exposure at various developmental stages of zebrafish has revealed that embryos and fry developed hepatic and mesenchymal tumors, while fry also developed testicular and blood vessel tumors. Juvenile zebrafish, however, demonstrated resistance to neoplastic transformation, suggesting stage-dependent sensitivity to carcinogens. Studies with DMBA resulted in liver neoplasms in zebrafish, closely resembling human liver cancer, further emphasizing zebrafish's potential for modeling human cancers. Additionally, treatment with Diethylnitrosamine (DEN) caused liver tumors and led to the translocation of the Maid protein, suggesting its role as a tumor suppressor. The role of polyploidy in cancer was explored using NDMA. Polyploid zebrafish showed delayed development of cholangiolar tumors, indicating that polyploidy might help prevent tumor suppressor gene inactivation, thus offering protection against certain cancers. Furthermore, the mutagen ENU has been used to generate mutant zebrafish lines with a predisposition to spontaneous neoplasms or heightened sensitivity to carcinogens. For instance, zebrafish with mutations in the Leucine Rich Repeat Containing 2 gene (lrrc50) developed seminomas, suggesting lrrc50 as a tumor suppressor gene. Similarly, mutant zebrafish lines developed testicular germ cell tumors, with carcinogen exposure exacerbating tumor development [19].

These findings further emphasize the zebrafish model’s potential in cancer research, particularly in understanding the genetic basis of tumor formation and identifying new therapeutic targets. Their ability to mimic human-like tumors across multiple organs makes zebrafish an ideal candidate for cancer modeling, drug testing, and personalized medicine approaches.

Arsenic-induced oxidative stress has been extensively studied as a key factor in arsenic-related carcinogenesis. The genotoxicity of reactive oxygen species (ROS) and reactive nitrogen species (RNS) plays a significant role in this process. Increasing evidence suggests that inorganic arsenic (iAs) and its methylated metabolites contribute to DNA damage, primarily through the generation of free radicals that indirectly harm DNA structures [20].

Arsenic's harmful effects extend across multiple organs, not only due to its direct toxicity but also because it is classified as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC). The carcinogenicity of

arsenic involves several mechanisms, including disruptions in DNA repair, chromosomal abnormalities, uncontrolled cell growth, and immune system dysregulation. While the exact pathophysiological processes by which arsenic induces cancer are still not fully understood, oxidative stress, chromosomal aberrations, and immune dysfunction are considered major contributors to arsenic-induced carcinogenesis. One of the key impacts of arsenic exposure is on DNA repair mechanisms, leading to oxidative DNA damage and mutations. This damage occurs due to the impairment of nucleotide excision repair, DNA ligase activity, base excision repair, and DNA strand break rejoining. In addition to direct DNA damage, arsenic exposure also affects epigenetic regulation. Research by Chanda et al. demonstrated that arsenic exposure leads to DNA hypermethylation in the promoter regions of critical tumor suppressor genes, such as p53 and p16. This epigenetic alteration can contribute to arsenic's carcinogenic effects by silencing genes crucial for controlling cell growth and apoptosis [21].

In conclusion, the carcinogenic effects of various substances, including arsenic, demonstrate the complexity of cancer development and the importance of model systems like zebrafish in studying these processes.

4. Conclusions and Future Directions

Zebrafish are becoming an increasingly important tool in cancer research because they offer several key benefits. They grow quickly, have clear bodies, share a lot of genetic similarities with humans, and can be easily altered genetically. These features make it easier to create cancer models and track tumor growth in real time, which speeds up research and helps scientists better understand cancer and explore possible treatments.

In conclusion, exposure to certain carcinogenic substances in zebrafish can significantly contribute to our understanding of tumor initiation and progression. The transparency of zebrafish larvae during early development provides a unique advantage, as it allows for real-time, non-invasive observation of tumor formation and growth. This characteristic enables researchers to monitor the effects of carcinogenic exposure directly, observing cellular and tissue-level changes as tumors develop. The zebrafish model, with its ability to visually track neoplastic transformation, offers a powerful tool in cancer research, facilitating the identification of key mechanisms involved in tumorigenesis and aiding in the evaluation of potential therapeutic interventions.

REFERENCES

[1] Nakayama, J., & Makinoshima, H. (2020). Zebrafish-Based Screening Models for the Identification of Anti-Metastatic Drugs. In Molecules (Vol. 25, Issue 10, p. 2407). MDPI AG. https://doi.org/10.3390/molecules25102407

[2] Hason, M., & Bartůněk, P. (2019). Zebrafish Models of Cancer-New Insights on Modeling Human Cancer in a Non-Mammalian Vertebrate. Genes, 10(11), 935. https://doi.org/10.3390/genes10110935
[3] Bootorabi, F., Manouchehri, H., Changizi, R., Barker, H., Palazzo, E., Saltari, A., Parikka, M., Pincelli, C., & Aspatwar, A. (2017). Zebrafish as a Model Organism for the Development of Drugs for Skin Cancer. In International Journal of Molecular Sciences (Vol. 18, Issue 7, p. 1550). MDPI AG. https://doi.org/10.3390/ijms18071550
[4] Bailey, J., Oliveri, A., & Levin, E. D. (2013). Zebrafish model systems for developmental neurobehavioral toxicology. In Birth Defects Research Part C: Embryo Today: Reviews (Vol. 99, Issue 1, pp. 14–23). Wiley. https://doi.org/10.1002/bdrc.21027
[5] Al-Thani, H. F., Shurbaji, S., & Yalcin, H. C. (2021). Zebrafish as a Model for Anticancer Nanomedicine Studies. In Pharmaceuticals (Vol. 14, Issue 7, p. 625). MDPI AG. https://doi.org/10.3390/ph14070625
[6] Feitsma, H., & Cuppen, E. (2008). Zebrafish as a Cancer Model. In Molecular Cancer Research (Vol. 6, Issue 5, pp. 685–694). American Association for Cancer Research (AACR). https://doi.org/10.1158/1541-7786.mcr-07-2167
[7] Siddiqui, I. A., Sanna, V., Ahmad, N., Sechi, M., & Mukhtar, H. (2015). Resveratrol nanoformulation for cancer prevention and therapy. In Annals of the New York Academy of Sciences (Vol. 1348, Issue 1, pp. 20–31). Wiley. https://doi.org/10.1111/nyas.12811
[8] Dupré, A., & Malik, H. Z. (2018). Inflammation and cancer: What a surgical oncologist should know. In European Journal of Surgical Oncology (Vol. 44, Issue 5, pp. 566–570). Elsevier BV. https://doi.org/10.1016/j.ejso.2018.02.209
[9] Dang, M., Fogley, R., & Zon, L. I. (2016). Identifying Novel Cancer Therapies Using Chemical Genetics and Zebrafish. In Advances in Experimental Medicine and Biology (pp. 103–124). Springer International Publishing. https://doi.org/10.1007/978-3-319-30654-4_5
[10] Shive HR. Zebrafish Models for Human Cancer. Veterinary Pathology. 2013;50(3):468-482. doi:10.1177/0300985812467471
[11] Amatruda, J. F., & Patton, E. E. (2008). Chapter 1 Genetic Models of Cancer in Zebrafish. In International Review of Cell and Molecular Biology (pp. 1–34). Elsevier. https://doi.org/10.1016/s1937-6448(08)01201-x
[12] White, R., Rose, K., & Zon, L. (2013). Zebrafish cancer: the state of the art and the path forward. In Nature Reviews Cancer (Vol. 13, Issue 9, pp. 624–636). Springer Science and Business Media LLC. https://doi.org/10.1038/nrc3589
[13] Spitsbergen, J. M., Tsai, H. W., Reddy, A., Miller, T., Arbogast, D., Hendricks, J. D., & Bailey, G. S. (2000). Neoplasia in zebrafish (Danio rerio) treated with 7,12-dimethylbenz[a]anthracene by two exposure routes at different developmental stages. Toxicologic pathology, 28(5), 705–715. https://doi.org/10.1177/019262330002800511
[14] Spitsbergen, J. M., Tsai, H. W., Reddy, A., Miller, T., Arbogast, D., Hendricks, J. D., & Bailey, G. S. (2000). Neoplasia in zebrafish (Danio rerio) treated with N-methyl-N'-nitro-N-nitrosoguanidine by three exposure routes at different developmental stages. Toxicologic pathology, 28(5), 716–725. https://doi.org/10.1177/019262330002800512
[15] Stern, H. M., & Zon, L. I. (2003). Cancer genetics and drug discovery in the zebrafish. In Nature Reviews Cancer (Vol. 3, Issue 7, pp. 533–539). Springer Science and Business Media LLC. https://doi.org/10.1038/nrc1126
[16] Gong, Z., Koh, C. H. V., Nguyen, A. T., Zhan, H., Li, Z., Lam, S. H., Spitsbergen, J. M., Emelyanov, A., & Parinov, S. (2010). The Zebrafish Model for Liver Carcinogenesis. In Molecular Genetics of Liver Neoplasia (pp. 197–218). Springer New York. https://doi.org/10.1007/978-1-4419-6082-5_11

[17] Shen, Y., Sheng, R., & Guo, R. (2023). Application of Zebrafish as a Model for Anti-Cancer Activity Evaluation and Toxicity Testing of Natural Products. In Pharmaceuticals (Vol. 16, Issue 6, p. 827). MDPI AG. https://doi.org/10.3390/ph16060827
[18] Zhao, S., Huang, J., & Ye, J. (2015). A fresh look at zebrafish from the perspective of cancer research. In Journal of Experimental & Clinical Cancer Research (Vol. 34, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1186/s13046-015-0196-8
[19] Cascallar, M., Alijas, S., Pensado-López, A., Vázquez-Ríos, A., Sánchez, L., Piñeiro, R., & de la Fuente, M. (2022). What Zebrafish and Nanotechnology Can Offer for Cancer Treatments in the Age of Personalized Medicine. In Cancers (Vol. 14, Issue 9, p. 2238). MDPI AG. https://doi.org/10.3390/cancers14092238
[20] Tam, L. M., Price, N. E., & Wang, Y. (2020). Molecular Mechanisms of Arsenic-Induced Disruption of DNA Repair. Chemical research in toxicology, 33(3), 709–726. https://doi.org/10.1021/acs.chemrestox.9b00464
[21] Huang, H.-W., Lee, C.-H., & Yu, H.-S. (2019). Arsenic-Induced Carcinogenesis and Immune Dysregulation. In International Journal of Environmental Research and Public Health (Vol. 16, Issue 15, p. 2746). MDPI AG. https://doi.org/10.3390/ijerph16152746