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Advances and challenges in zebrafish models of T-cell acute lymphoblastic leukemia

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ABSTRACT

T-cell acute lymphoblastic leukemia (T-ALL) presents a significant challenge in oncology, necessitating innovative research approaches for improved comprehension and therapeutic advancements. In recent years, zebrafish (Danio rerio) have emerged as a vital model organism in T-ALL research, conferring distinctive advantages over conventional models. Zebrafish, sharing substantial genetic homology with humans, accurately replicate key phenotypic and molecular features of human T-ALL, including clonal expansion, aneuploidy, organ infiltration, and gene expression profiles. They also develop chromosomal translocations similar to those in human T-ALL. This comprehensive review explores the diverse zebrafish T-ALL models, encompassing genetic alterations and inducible systems that facilitate the investigation of disease initiation, progression, and response to therapy. These models have revealed critical insights into the roles of specific genes and the identification of novel oncogenic drivers. Moreover, zebrafish models enable high-throughput drug screening, providing an expedited path toward discovering potential therapeutics. However, while zebrafish models present exceptional advantages, they are not without challenges, including genetic differences, biological disparities, and technical complexities. To exploit the potential of zebrafish in T-ALL research, ongoing efforts are directed toward CRISPR/Cas9 gene editing, improved antibody development, and enhanced experimental techniques.

KEYWORDS

Zebrafish; Gene editing; T-cell lymphoblastic leukemia; Transcriptome; CRISPR-Cas; Aneuploidy; T-lymphocytes

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Introduction

T-cell acute lymphoblastic leukemia (T-ALL) is a rare and aggressive type of blood cancer characterized by the abnormal proliferation of immature T cells [1]. T-ALL accounts for about 10-15% of childhood and 25% of adult cases of acute lymphoblastic leukemia (ALL), with a poor prognosis and high relapse rate [2]. Understanding the molecular mechanisms and genetic factors that drive T-ALL development and progression is crucial for improving the diagnosis and treatment of this disease. In recent years, animal models, particularly the zebrafish (Danio rerio), have emerged as invaluable tools for this purpose.

Cancer research has long relied on animal models to bridge the gap between in vitro experiments and clinical applications. These models provide a dynamic platform to investigate the interplay of genetic, environmental, and therapeutic factors within a physiologically relevant context [3]. In leukemia research, animal models are crucial for understanding the molecular events driving disease initiation, progression, and response to treatment [4]. Furthermore, they allow for exploing intricate interactions between leukemia cells and the microenvironment, shedding light on the complexities of tumor-host interactions. For instance, transgenic zebrafish models expressing human oncogenes have been developed to study the early events in T-ALL pathogenesis, providing insights into the roles of specific oncogenes in disease initiation and progression [5].

The zebrafish is an exceptional model organism for studying leukemia due to its rapid development, transparency during embryogenesis, and genetic manipulability make it uniquely suited for studying cancer's cellular and molecular events in real time. Importantly, zebrafish share significant genetic homology with humans, particularly in oncogenes, making them a reliable proxy for investigating disease mechanisms and therapeutic targets [2].

This comprehensive review aims to address these research gaps and challenges associated with utilizing zebrafish models in studying T-ALL. The primary objectives include summarizing recent discoveries in T-ALL research using zebrafish models, evaluating the limitations and challenges in employing these models for studying T-ALL and further identifying potential future directions and applications of zebrafish models in T-ALL research. While remarkable progress has been made in T-ALL research, several critical questions and knowledge gaps remain. This review will critically examine the existing evidence, provide insights into possible resolutions, and explore how zebrafish models can resolve these issues.

Zebrafish as a Model Organism in T-ALL Research

Zebrafish (Danio rerio) is a valuable model organism in developmental biology and cancer research, share approximately 70% of human genes, and possess orthologues for over 80% of disease-associated human proteins [6]. This genetic constitution makes zebrafish unique for studying a wide spectrum of human diseases, particularly T-ALL.

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Advantages of zebrafish T-ALL models

Due to their unique advantages, the zebrafish T-ALL models set them apart from conventional animal models like mice and rats.

1. **Cost-effectiveness and scalability:** zebrafish's small size and prolific breeding make them an economical choice for large-scale experiments. [7].
2. **Transparency for real-time monitoring:** zebrafish embryos and larvae possess translucency, enabling real-time visualization of T-ALL development and progression, providing an advantage over other models [8].
3. **Genetic manipulation:** zebrafish are amenable to genetic manipulation, facilitating the generation of models that can replicate specific genetic mutations/alterations implicated in human T-ALL [9].
4. **High-throughput drug screening:** zebrafish can be employed in high-throughput drug screens, accelerating the identification of potential T-ALL therapies [10].

Generating zebrafish T-ALL models

Several strategies can be employed to generate zebrafish T-ALL models, each providing distinct perspectives on the disease:

1. **Manipulation of oncogenes and tumor suppressors:** transgenic zebrafish or CRISPR/Cas9 gene editing can be employed to overexpress or knock down oncogenes and tumor suppressors, mimicking genetic alterations found in human T-ALL [11].
2. **Chemical or radiation mutagenesis:** random mutations can be induced using chemical or radiation mutagenesis, resulting in a diverse pool of mutants that can be screened to identify potential T-ALL genes [12].
3. **Human T-ALL cell transplantation:** zebrafish embryos or adults can be transplanted with human T-ALL cells, investigating the interactions between these cells and the zebrafish microenvironment [11].

Zebrafish T-ALL Models Replicate Human Phenotypes

Zebrafish T-ALL models replicate phenotypic and molecular features of human T-ALL, which are essential for studying the disease's mechanisms and progression:

1. **Clonal expansion:** T-ALL cells in zebrafish models exhibit uncontrolled proliferation, forming clonal tumors resembling human T-ALL [13].
2. **Aneuploidy:** zebrafish T-ALL cells frequently exhibit abnormal chromosome numbers, paralleling a characteristic feature of human T-ALL [13].
3. **Organ infiltration:** T-ALL cells in zebrafish models can infiltrate various organs, including the liver, spleen, and thymus, mimicking the tissue involvement observed in human T-ALL patients [13].
4. **Gene expression profiles:** zebrafish T-ALL cells manifest gene expression profiles closely reflecting their human counterparts, facilitating investigations into disease-specific pathways [13].
5. **Chromosomal translocations:** some zebrafish T-ALL models develop chromosomal translocations similar to those found in human T-ALL patients, providing a platform to study the consequences of these genetic aberrations [13].

Different zebrafish models of T-ALL

The zebrafish models encompass a range of genetic alterations and inducible systems, providing valuable insights into T-ALL initiation, progression, and response to therapy. These models include those driven by the potent oncogene Myc (Models 1 and 2), revealing the critical role of Myc in T-ALL and its utility for large-scale compound screens. Conditional models (Model 2) and inducible models (Model 4) have revealed interactions between Myc, Notch1, PTEN, and AKT, uncovering potential therapeutic targets. Chemical mutagenesis screens (Model 6) have identified genetic predispositions to T-ALL, allowing for the exploration of somatic mutations and their clinical relevance. Additionally, models driven by other genes, such as ARID5B (Model 9) and JDP2 (Model 10), have unveiled novel oncogenic drivers and mechanisms of glucocorticoid resistance. These zebrafish models provide a powerful platform for understanding the complexities of T-ALL, shown in Table 1, offering opportunities to study disease progression, test potential therapeutics, and investigate genetic interactions. Their contributions have advanced our knowledge of T-ALL pathogenesis and hold promise for developing targeted therapies and improved patient outcomes [3, 13-19].

Table 1. Various zebrafish models used in T-all research, highlighting their phenotypes, methods of transgenesis, key discoveries, pathways implicated, and the cell line or transgenes used.

Model	Phenotypes	Method of transgenesis	Key discoveries	Pathways implicated	Cell line/trans-genes used
Model 1 (Tg(zrag2:EGFP-mMyc) Zebrafish) [13]	Rapid T-ALL onset Complete penetrance Clonal aneuploid T-ALL Immortality of T-ALL Resembles human T-ALL	Microinjection of zrag2:EGFP-mMyc transgene	MYC's role as a potent oncogene Resemblance to common human T-ALL subtype Role of DLST and TCA in Metabolism of T-ALL Creation of transplantable T-ALL cell line (ZL1)	MYC, TAL1/SCL, LMO2, DLST, TCA Metabolism	zrag2:EGFP-mMyc

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Model 2a (rag2:loxP-dsRED2-loxP-EGFP-mMyc)[14]	Conditional expression of EGFP-mMyc Longer T-ALL latency Mosaic EGFP-mMyc activation	Conditional Cre-Lox technology with loxP-DsRed2-loxP cassette	Role of UFD1 in MYC-driven T-ALL	MYC, DLST, TCA Metabolism	rag2:loxP-dsRED2-loxP-EGFP-mMyc
Model 2b (hsp70:Cre;rag2:LDL-EMyc) [15]	Improved T-ALL penetrance Heat shock-inducible Cre recombinase	Heat shock-inducible Cre transgene (hsp70:Cre)	Improved T-ALL penetrance AKT's role in T-LBL progression to T-ALL	MYC, UFD1	Tg(hsp70:Cre;rag2:LDL-EMyc)
Model 2c (hsp70:Cre;rag2:LDL-EMyc;rag2:EGFP-bcl2)[3]	Accelerated T-LBL onset Constitutive bcl2 overexpression Inhibited T-LBL progression to T-ALL	Triple-transgenic fish with hsp70:Cre, rag2-LDL-EGFP-mMyc, and rag2:EGFP-bcl2	Autophagy's role in T-LBL progression S1P1 signaling and ICAM1's role in T-LBL dissemination	BCL2, S1P1, ICAM1	Tg(hsp70:Cre;rag2:LDL-EMyc;rag2:EGFP-bcl2)
Model 3 (Co-injection Strategies)[16]	Various transgene combinations Rapid assessment of gene synergy/antagonism	Co-injection of multiple transgenes simultaneously	Quantifying leukemia-initiating cells (LIC) Stochastic acquisition of mutations leading to treatment resistance	Variable depending on co-injected transgenes	Various combinations of transgenes
Model 4 (rag2:hMYC-ER Transgenic Zebrafish) [17]	Inducible MYC activation Reversible MYC activation Delayed T-ALL onset without 4HT T-ALL occurrence without 4HT	hMYC fused to an estrogen receptor (ER)	Role of PI3K-AKT pathway in T-ALL BIM repression as a key event in treatment-resistant T-ALL Identification of PP2A as a therapeutic target	MYC, PI3K-AKT, PTEN	rag2:hMYC-ER
Model 5a (Transgenic Human NOTCH1–Tg(rag2:hICN1-EGFP) Zebrafish)[3]	NOTCH1-driven T-ALL Low penetrance Shared molecular signature with human T-ALL	Expression of hICN1 (constitutively active NOTCH1) fused to EGFP	NOTCH activation as an initiating event in human T-ALL Cooperation between NOTCH1 and BCL2	NOTCH1, BCL2	Tg(rag2:hICN1-EGFP)
Model 5b (Transgenic Zebrafish Notch1–Tg(rag2:znotch1aICD))[3]	Cooperation between NOTCH1 and MYC Similar LIC frequencies with or without NOTCH1	Expression of znotch1aICD (truncated NOTCH1)	NOTCH1 as an initiating event in human T-ALL Shared molecular signature in T-ALL across species	NOTCH1, MYC	Tg(rag2:znotch1aICD), rag2:mMyc

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Models 6–8 (Non-Transgenic T-ALL Models) [3]	Chemically induced T-ALL Different germline mutations in hlk, srk, otg Copy number aberrations shared with human T-ALL	Chemical mutagenesis with N-ethyl-N-nitrourea (ENU)	Identification of non-transgenic T-ALL models Exploration of somatically acquired genetic changes in T-ALL	Varied, complex genetic alterations	Non-transgenic fish lines with unidentified germline mutation
Model 9 (T-ALL Induced by ARID5B–Tg(rag2:hARID5B) Zebrafish)[18]	Delayed thymic involution Resistance to glucocorticoids Low T-ALL penetrance High MCL1 expression	Expression of hARID5B	Oncogenic role of ARID5B in T-ALL Link between ARID5B and MCL1	ARID5B, MYC, TAL1/SCL, MCL1	Tg(rag2:hARID5B)
Model 10 (T-ALL Induced by jdp2–Tg(rag2:zjdp2) Zebrafish)[3]	Accelerated T-ALL onset with JDP2 overexpression Resistance to glucocorticoids Thymic hyperplasia	Expression of zjdp2 (JDP2)	Oncogenic role of JDP2 in T-ALL Regulation of MCL1 by JDP2	JDP2, MCL1	Tg(rag2:zjdp2)

Advancements in zebrafish models of T-ALL

Generation and Characterization: Zebrafish T-ALL models can be created through transgenic, mutagenic, or xenotransplantation approaches. These models faithfully replicate phenotypic and molecular features of human T-ALL, such as clonal expansion, aneuploidy, organ infiltration, gene expression profiles, and chromosomal translocations [19].

Identification of Novel Genes and Pathways: Zebrafish T-ALL models aid in identifying and validating novel T-ALL-related genes and pathways. Techniques like forward genetic screens and reverse genetic approaches (CRISPR/Cas9 or knockdown) elucidate the functional roles of genes like notch1a, rag1, lmo2, and others, shedding light on T cell development, differentiation, survival, proliferation, and apoptosis [20].

Drug Discovery and Testing: Zebrafish models offer advantages over conventional animals for drug discovery. Zebrafish embryos allow high-throughput screening of compounds targeting T-ALL-associated pathways or genes. Adult zebrafish mimic human pharmacokinetics and can assess drug efficacy and toxicity in vivo. Xenotransplantation models enable testing drugs on human T-ALL cells in live hosts [21].

Challenges and Limitations of Zebrafish Models of T-ALL

Zebrafish models of T-ALL have unique advantages over traditional animal models. However, these models come with their challenges and limitations, which must be carefully considered when interpreting experimental data.

Challenges

Zebrafish as a model organism presents several challenges. Research involving zebrafish as a model organism presents several challenges. Despite significant genetic homology shared between zebrafish and humans, zebrafish possess a teleost genome duplication, leading to gene redundancy. This genetic divergence can lead to difficulty in determining which gene copies are functionally relevant in studies [22]. Additionally, zebrafish T-ALL cells exhibit higher resistance to apoptosis compared to human T-ALL cells, creating disparities in cellular responses and potentially limiting the applicability of findings to human diseases. Another obstacle lies in the lack of commercially available high-quality antibodies specific to zebrafish proteins, making techniques such as immunohistochemistry and western blotting challenging. Moreover, the small and delicate nature of zebrafish embryos and larvae demands specialized skills for procedures like microinjections and transplantation, adding to the technical challenges faced by researchers in this field [22,23].

Limitations

Understanding the diversity of human T-cell acute lymphoblastic leukemia (T-ALL) at a genetic and molecular level is crucial, as T-ALL exhibits significant heterogeneity. Attempting to capture this complexity using a single zebrafish T-ALL model is unlikely to be comprehensive. Therefore, employing multiple zebrafish models becomes essential for conducting thorough and meaningful T-ALL studies. While zebrafish models provide valuable insights, it is important to note that their predictive value for human therapeutic responses is limited. Therapies that show promise in zebrafish T-ALL models may not necessarily perform similarly in human patients. Consequently, any findings derived from zebrafish models must undergo rigorous validation through preclinical and clinical trials to ensure their applicability and effectiveness in human T-ALL treatment strategies [24].

Future prospects

Advanced genetic manipulation, antibody development, and improved experimental techniques enhance the utility of zebrafish models for cancer research. Researchers are actively

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developing innovative approaches. In cancer research, advanced techniques transform the study of diseases like T-cell Acute Lymphoblastic Leukemia (T-ALL). CRISPR/Cas9 gene editing enables the creation of zebrafish models mirroring human T-ALL, introducing specific genetic mutations for a closer resemblance to the human disease [25]. Efforts are ongoing to develop antibodies that effectively target zebrafish proteins, crucial for techniques like Western blotting and Immunohistochemistry, deepening our understanding of T-ALL biology in zebrafish [26]. Experimental methods involving zebrafish embryos and larvae are continually refined, enhancing procedures such as microinjection and transplantation, leading to significant progress in cancer research [27].

Conclusions

Zebrafish (Danio rerio) has emerged as an efficient model for studying T-cell acute lymphoblastic leukemia (T-ALL), a severe blood cancer. Their genetic similarity to humans, small size, prolific breeding, embryonic transparency, and genetic manipulability make them cost-effective and scalable for research. Zebrafish models accurately replicate key features of human T-ALL, including clonal expansion, aneuploidy, organ infiltration, gene expression profiles, and chromosomal translocations. They have been engineered to mimic specific genetic mutations implicated in human T-ALL, illuminating critical oncogenes, tumor suppressors, and pathways. Despite challenges such as genetic redundancy and technical demands, advancements like CRISPR/Cas9 gene editing enhance their utility. Zebrafish models have led to pivotal discoveries in T-ALL research and hold promise for discovering novel therapeutic targets to improve patient outcomes. As we navigate the advances and challenges in this evolving field, the synergy between zebrafish research and clinical innovation can revolutionize the T-ALL treatment strategies, offering a resolution for patients and advancing oncology research.

Disclosure statement

No potential conflict of interest was reported by the author.

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