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Modeling Human Disease and Development in Zebrafish

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Many of the critical pathways that govern vertebrate development are highly conserved between humans and zebrafish (*Danio rerio*). The zebrafish genome shares a high degree of sequence similarity to that of humans. Approximately 70% of genes associated with diseases in humans have functional homologs in the zebrafish [1]. In addition zebrafish as an experimental model offers many advantages including their ability to produce large number of eggs (a single cross can generate 200–300 embryos), they develop outside the body, are transparent making them amenable to follow during organogenesis. Development is rapid with major organ primordial forming by 24 hours after fertilization. Compared to other vertebrate models, zebrafish are easy and inexpensive to raise and maintain. [2]. George Streisinger, a founding father of zebrafish research was one of the first to work with zebrafish in the late 1960s [3]. He began to study embryonic development particularly that of the nervous system by employing mutant strains [4-6]. Realizing the importance of the zebrafish model, Grunwald and Eisen used this developmental model to study the segmental structures of the brain and characterized neurons in the zebrafish that had not been reported in any other vertebrate model [7]. Christiane Nüsslein-Volhard, a fruit fly geneticist at the University of Tübingen, who identified 120 developmentally important genes in *Drosophila melanogaster* [8], recognized the usefulness of zebrafish as a vertebrate model, and established collaboration with Marc Fishman at the Massachusetts General Hospital [9] to study these developmentally important genes in zebrafish.

Forward Genetics

In Forward Genetics heritable mutagenic lesions are created with the use of chemical (*ENU: Nethyl- N-nitrosourea*) [9] or insertional (retroviruses or transposons) [10,11] mutagenesis approaches. Mutagenic lesions are screened for particular phenotypes and the causative genes responsible for a given phenotype are identified through positional cloning and or through the candidate gene approach [12,13]. The success of the "Big forward genetic screen" Nüsslein-Volhard et al. [9] commonly referred as "*Tübingen/Boston screens*" created a significant impact on the use of zebrafish as a promising system to model disease and development. The results were published in the entire volume of the Journal Development [14]. A major drawback of this approach is, with the large size of the zebrafish genome the identification of mutant genes can be time consuming and laborious [15].

Reverse Genetics

Reverse Genetics involves the selection of a target gene and creation of mutants of the selected gene and investigation of the associated phenotypes to uncover function of the gene in question. Many reverse genetic approaches have been developed recently [16]. These include the use of antisense morpholino (MO) oligonucleotide mediated gene knockdown technology [17,18], Targeting induced local lesions in genome (Tilling) [16,19], Zinc Finger Nucleases (ZFN) [20,21], Transcription Activator-Like Effector Nucleases (TALENs) [22] Tol2 mediated Transgenesis [23,24], GAL4-UAS System [25], Tol2-mediated Gal4/UAS [26], Cre/Lox system [27] and a tamoxifen-inducible Cre/lox method [28]. Among them TALENs, ZFNs and Tol2 mediated transgenesis methods are becoming successful in defining the functional roles of target genes. Both GAL4/UAS and Cre/Lox methods are less efficient due to the limited understanding of tissue/cell specific promoters and there is no guarantee that they will work as expected. Further development in these technologies would facilitate their better application in zebrafish research.

Modeling Vertebrate Development

Both forward and reverse genetic approaches have been employed in zebrafish to define the role of genes involved in the development of vertebrate organs, tissues and cells. A recent key word search of PubMed revealed 8596 publications on the use of zebrafish in vertebrate development. Some of the examples include development of the cardiovascular system [29], the endoderm [30], motor neurons [31,32] and craniofacial structures [33]. The insights gained from zebrafish are directly applicable to humans since molecular mechanisms that regulate vertebrate development are highly conserved between the two species.

Modeling Disease

With the exception of few organs namely the lungs, prostrate and mammary gland, most of the tissues and organs present in humans are found in the zebrafish. The cloning of mutated genes screened for specific phenotypes have revealed similarity in humans and thus serves as models for human disease and to study underlying mechanisms. The first human disease model in zebrafish to be defined in this was the sauternes (*sau*) mutant responsible for a blood disorder involving a specific defect in hemoglobin production. The mutated gene responsible for the blood disorder was ALAS-2. Many other mutants showing phenotypic similarity to human diseases have been screened and later identified to have human homologues. These include hematological disorders [34,35], neurological disorders [36], cardiovascular diseases [37], muscle disease [38] and cancers [39,40]. Detailed reviews on modeling human diseases in zebrafish have been published [1,7,41,42].

Drug Screening

High throughput chemical screening in zebrafish is a very promising tool since it can be undertaken by simply placing zebrafish embryos in 96 well plates, adding chemicals to the water and then looking for

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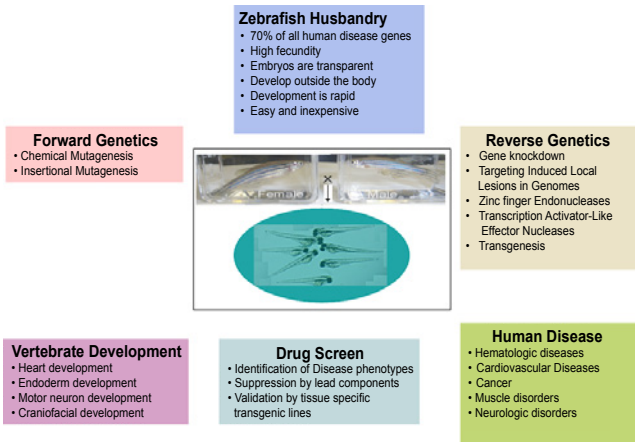

Figure 1: Zebrafish as a Genetic and Developmental Model. Zebrafish has emerged as a powerful system to model vertebrate disease and development using various molecular and genetic tools. Zebrafish genome possesses a high degree of similarity to that of humans. The other advantages of using the zebrafish model include the generation of large numbers of embryos per cross, transparency and development outside the body. Development is rapid, and embryos are comparatively easy and inexpensive to grow and maintain. The success of the "Big forward genetic screen" commonly referred as "Tübingen/ Boston screens" generated significant impact on the use of zebrafish as a promising system to model disease and development. Recent establishment of various reverse genetic approaches as listed in the above figure serves as a promising tool to interrogate the function of target genes. Application of both forward and reverse genetic approaches in the zebrafish model will uncover the roles and biological functions of many genes involved and to gain insights about the associated signaling pathways during development including namely heart and craniofacial development and disease processes including blood disorders, cardiovascular diseases and cancer. The use of zebrafish in the drug screening approach potentially facilitates fast and cost-effective identification of potential lead components and initial validation as potential therapeutics.

the suppression of a given phenotype. The efficacy and toxicity of new compounds can be simultaneously assessed by employing this method [43]. The applicability and advantages of using zebrafish embryos over other models in drug screening have reviewed in detail [44-46]. Large scale chemical screening using zebrafish have been conducted recently with the aim of identifying novel biological and therapeutic compounds [47]. Important compounds discovered in this way are being tested currently in clinical trials [48,49].

Conclusion

Zebrafish is a versatile system, offering many molecular and genetic tools to model human disease and development to study gene function during normal development and disease. More recently its utility in the identification of lead compounds by drug screening has proven to be cost and time effective. However, potential therapeutic compounds identified from initial screening need to be further validated using another vertebrate model systems before making final decisions about the possible development of the compound for use in treating particular diseases (Figure 1).

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References

1. Santoriello C, Zon LI (2012) Hooked! Modeling human disease in zebrafish. J Clin Invest 122: 2337-2343.
2. Lieschke GJ, Currie PD (2007) Animal models of human disease: zebrafish swim into view. Nat Rev Genet 8: 353-367.
3. Stahl FW (1985) George Streisinger (December 27, 1927-August 11, 1984). Genetics 109: 1-2.
4. Chakrabarti S, Streisinger G, Singer F, Walker C (1983) Frequency of gamma-Ray Induced Specific Locus and Recessive Lethal Mutations in Mature Germ Cells of the Zebrafish, BRACHYDANIO RERIO. Genetics 103: 109-123.
5. Streisinger G, Singer F, Walker C, Knauber D, Dower N (1986) Segregation analyses and gene-centromere distances in zebrafish. Genetics 112: 311-319.
6. Walker C, Streisinger G (1983) Induction of Mutations by gamma-Rays in Pregonial Germ Cells of Zebrafish Embryos. Genetics 103: 125-136.
7. Grunwald DJ, Eisen JS (2002) Headwaters of the zebrafish -- emergence of a new model vertebrate. Nat Rev Genet 3: 717-724.
8. Nüsslein-Volhard C, Wieschaus E (1980) Mutations affecting segment number and polarity in Drosophila. Nature 287: 795-801.
9. Mullins MC, Hammerschmidt M, Haffter P, Nüsslein-Volhard C (1994) Large-scale mutagenesis in the zebrafish: in search of genes controlling development in a vertebrate. Curr Biol 4: 189-202.
10. Gaiano N, Amsterdam A, Kawakami K, Allende M, Becker T, et al. (1996) Insertional mutagenesis and rapid cloning of essential genes in zebrafish. Nature 383: 829-832.
11. Sivasubbu S, Balciunas D, Davidson AE, Pickart MA, Hermanson SB, et al. (2006) Gene-breaking transposon mutagenesis reveals an essential role for histone H2afza in zebrafish larval development. Mech Dev 123: 513-529.
12. Gates MA, Kim L, Egan ES, Cardozo T, Sirotkin HI, et al. (1999) A genetic linkage map for zebrafish: comparative analysis and localization of genes and expressed sequences. Genome Res 9: 334-347.
13. Woods IG, Wilson C, Friedlander B, Chang P, Reyes DK, et al. (2005) The zebrafish gene map defines ancestral vertebrate chromosomes. Genome Res 15: 1307-1314.
14. Haffter P, Granato M, Brand M, Mullins MC, Hammerschmidt M, et al. (1996) The identification of genes with unique and essential functions in the development of the zebrafish, Danio rerio. Development 123: 1-36.
15. Flicek P, Amode MR, Barrell D, Beal K, Brent S, et al. (2011) Ensembl 2011. Nucleic Acids Res 39: D800-806.
16. Lawson ND, Wolfe SA (2011) Forward and reverse genetic approaches for the analysis of vertebrate development in the zebrafish. Dev Cell 21: 48-64.
17. Nasevicius A, Ekker SC (2000) Effective targeted gene 'knockdown' in zebrafish. Nat Genet 26: 216-220.
18. Chitramuthu BP, Bennett HP (2011) Use of zebrafish and knockdown technology to define proprotein convertase activity. Methods Mol Biol 768: 273-296.
19. Wienholds E, Schulte-Merker S, Walderich B, Plasterk RH (2002) Target-selected inactivation of the zebrafish rag1 gene. Science 297: 99-102.
20. Kim YG, Cha J, Chandrasegaran S (1996) Hybrid restriction enzymes: zinc finger fusions to Fok I cleavage domain. Proc Natl Acad Sci U S A 93: 1156-1160.
21. Doyon Y, McCammon JM, Miller JC, Faraji F, Ngo C, et al. (2008) Heritable targeted gene disruption in zebrafish using designed zinc-finger nucleases. Nat Biotechnol 26: 702-708.
22. Moscou MJ, Bogdanove AJ (2009) A simple cipher governs DNA recognition by TAL effectors. Science 326: 1501.
23. Kawakami K (2007) Tol2: a versatile gene transfer vector in vertebrates. Genome Biol 8: S7.
24. Suster ML, Kikuta H, Urasaki A, Asakawa K, Kawakami K (2009) Transgenesis in zebrafish with the tol2 transposon system. Methods Mol Biol 561: 41-63.
25. Schlueter PJ, Peterson RT (2009) Systematizing serendipity for cardiovascular drug discovery. Circulation 120: 255-263.
26. Asakawa K, Kawakami K (2008) Targeted gene expression by the Gal4-UAS system in zebrafish. Dev Growth Differ 50: 391-399.
27. Hans S, Kaslin J, Freudenreich D, Brand M (2009) Temporally-controlled site-specific recombination in zebrafish. PLoS One 4: e4640.
28. Stewart S, Stankunas K (2012) Limited dedifferentiation provides replacement tissue during zebrafish fin regeneration. Dev Biol 365: 339-349.

29. Quaife NM, Watson O, Chico TJ (2012) Zebrafish: an emerging model of vascular development and remodelling. Curr Opin Pharmacol 12: 608-614.

30. Xu C, Fan ZP, Müller P, Fogley R, DiBiase A, et al. (2012) Nanog-like regulates endoderm formation through the Mxtx2-Nodal pathway. Dev Cell 22: 625-638.

31. Feldner J, Reimer MM, Schweitzer J, Wendik B, Meyer D, et al. (2007) PlexinA3 restricts spinal exit points and branching of trunk motor nerves in embryonic zebrafish. J Neurosci 27: 4978-4983.

32. Chitramuthu BP, Baranowski DC, Kay DG, Bateman A, Bennett HP (2010) Progranulin modulates zebrafish motoneuron development in vivo and rescues truncation defects associated with knockdown of Survival motor neuron 1. Mol Neurodegener 5: 41.

33. Jayasena CS, Bronner ME (2012) Rbms3 functions in craniofacial development by posttranscriptionally modulating TGF-β signaling. J Cell Biol 199: 453-466.

34. Berman J, Payne E, Hall C (2012) The zebrafish as a tool to study hematopoiesis, human blood diseases, and immune function. Adv Hematol 2012: 425345.

35. Brownlie A, Donovan A, Pratt SJ, Paw BH, Oates AC, et al. (1998) Positional cloning of the zebrafish sauternes gene: a model for congenital sideroblastic anaemia. Nat Genet 20: 244-250.

36. Gama Sosa MA, De Gasperi R, Elder GA (2012) Modeling human neurodegenerative diseases in transgenic systems. Hum Genet 131: 535-563.

37. Sehnert AJ, Huq A, Weinstein BM, Walker C, Fishman M, et al. (2002) Cardiac troponin T is essential in sarcomere assembly and cardiac contractility. Nat Genet 31: 106-110.

38. Lin YY (2012) Muscle diseases in the zebrafish. Neuromuscul Disord 22: 673-684.

39. Liu S, Leach SD (2011) Zebrafish models for cancer. Annu Rev Pathol 6: 71-93.

40. Patton EE, Widlund HR, Kutok JL, Kopani KR, Amatruda JF, et al. (2005) BRAF mutations are sufficient to promote nevi formation and cooperate with p53 in the genesis of melanoma. Curr Biol 15: 249-254.

41. Zon LI (1999) Zebrafish: a new model for human disease. Genome Res 9: 99-100.

42. Kari G, Rodeck U, Dicker AP (2007) Zebrafish: an emerging model system for human disease and drug discovery. Clin Pharmacol Ther 82: 70-80.

43. Hao J, Ho JN, Lewis JA, Karim KA, Daniels RN, et al. (2010) In vivo structure-activity relationship study of dorsomorphin analogues identifies selective VEGF and BMP inhibitors. ACS Chem Biol 5: 245-253.

44. Wheeler GN, Brändli AW (2009) Simple vertebrate models for chemical genetics and drug discovery screens: lessons from zebrafish and Xenopus. Dev Dyn 238: 1287-1308.

45. Kaufman CK, White RM, Zon L (2009) Chemical genetic screening in the zebrafish embryo. Nat Protoc 4: 1422-1432.

46. Delvecchio C, Tiefenbach J, Krause HM (2011) The zebrafish: a powerful platform for in vivo, HTS drug discovery. Assay Drug Dev Technol 9: 354-361.

47. Tan JL, Zon LI (2011) Chemical screening in zebrafish for novel biological and therapeutic discovery. Methods Cell Biol 105: 493-516.

48. North TE, Goessling W, Walkley CR, Lengerke C, Kopani KR, et al. (2007) Prostaglandin E2 regulates vertebrate haematopoietic stem cell homeostasis. Nature 447: 1007-1011.

49. White RM, Cech J, Ratanasirintrawoot S, Lin CY, Rahl PB, et al. (2011) DHODH modulates transcriptional elongation in the neural crest and melanoma. Nature 471: 518-522.