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Workshop report: The medaka model for comparative assessment of human disease mechanisms

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ABSTRACT

Results of recent studies showing the utility of medaka as a model of various human disease states were presented at the 7th Aquatic Models of Human Disease Conference (December 13-18, 2014, Austin, TX). This conference brought together many of the most highly regarded national and international scientists that employ the medaka model in their investigations. To take advantage of this opportunity, a cohort of established medaka researchers were asked to stay an extra day and represent the medaka scientific community in a workshop entitled "The Medaka Model for Comparative Assessment of Human Disease Mechanisms."

The central purpose of this medaka workshop was to assess current use and project the future resource needs of the American medaka research community. The workshop sought to spur discussions of issues that would promote more informative comparative disease model studies. Finally, workshop attendees met together to propose, discuss, and agree on recommendations regarding the most effective research resources needed to enable US scientists to perform experiments leading to impacting experimental results that directly translate to human disease.

Consistent with this central purpose, the workshop was divided into two sessions of invited speakers having expertise and experience in the session topics. The workshop hosted 20 scientific participants (Appendices 1 and 2), and of these, nine scientists presented formal talks.

Here, we present a summary report stemming from workshop presentations and subsequent round table discussions and forward recommendations from this group that we believe represent views of the overall medaka research community.

1. Introduction to the medaka model

The Japanese medaka (*Oryzias latipes*) has a scientific history dating back to 1921 (Aida, 1921). Zebrafish and medaka are among the most studied teleost experimental models employed in biomedical research. Medaka is utilized worldwide and substantial resources are available to researchers, such as a fully sequenced genome, high-resolution genetic maps, inbred lines, hundreds of mutants, independently derived wild stocks, transgenic knock in and knock out capabilities, and many others.

Medaka occur naturally in Japan, Korea, and China, where animals are available in the wild and possess naturally occurring variation that is likely to be relevant to human disease studies. An example of this strain variation in relation to personalized medicine involves the recent development of transgenic medaka from several different genetic backgrounds that all carry the same melanoma driver gene from *Xiphophorus* (*Xmrk2*: Schartl et al., 2010 and unpublished). In this case, the same driver gene construct led to the development of different tumor types in each of the three varied medaka genetic backgrounds. This natural variation among medaka is being exploited at the Karlsruhe Institute of Technology (Germany), where a population genetics resource is being produced utilizing 150 medaka lines derived from widely variant wild populations (Spivakov et al, 2014). Once this system is operational, scientists may assess the effects of the variable genetic backgrounds on any driver gene (e.g., oncogene, transcription factor, etc.) (see Kirchmaier et al., 2015). There is no resource such as this available for the any other vertebrate aquatic model.

Medaka, like zebrafish, is oviparous and has a clear chorion allowing easy visualization of all stages of early development, from the single cell to the free-swimming hatchling. "See through" transparent medaka lines lack all pigment and thus allow fluorescent visualization of gene expression within the living animal at any developmental stage (Wakamatsu et al., 2001). From an evolutionary viewpoint, medaka is more closely related to several other commonly utilized experimental fish models (i.e., *Xiphophorus*, Stickleback, *Fugu*, *Fundulus*, etc.) (Kitambi and Malicki, 2008; Ichimura et al., 2012, 2013) than zebrafish. Medaka possesses a small genome (700 Mb, less than half the size of the

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zebrafish genome) represented on 24 chromosome pairs that largely maintain ancestral vertebrate syntenic relationships present throughout the vertebrate classes, including to humans (Naruse et al., 2004). The small genome of medaka makes identification of regulatory sequences more convenient than in animals with larger genomes. Inbred medaka lines are available for fine mapping of complex trait loci and for detailed genetic dissection of human disease models.

Due to the use of medaka as an experimental model in Japan, the medaka genome was one of the first small fish genomes sequenced and assembled. The medaka genome project began in 2000 and was aided by the sequence assembly of the first fish genome, *Fugu*, in 2002 since medaka and *Fugu* are evolutionarily close to each other (Kasahara et al, 2007). However, given the early sequencing of the medaka genome, the medaka assembly could be vastly and quickly improved with the application of contemporary technologies. The National Institute of Basic Biology, University of Tokyo, maintains a very impressive medaka resource center for both medaka fish and experimental resources related to research use of medaka (http://www.shigen.nig.ac.jp/medaka/). However, due to post-September 11, 2001 restrictions on international shipping of animals and other resources, it is challenging for American scientists to gain access and utilize many of the resources available in Japan.

The increasing use of medaka in the development of human disease models, the topic of this workshop, hallmarks a renewed scientific interest in medaka in the USA. Presentations at the workshop documented medaka as a valuable comparative model, with the zebrafish, but also, that many newly developed medaka disease models were able to provide more direct translational understating of the human condition for diseases such as osteoporosis (To et al, 2012), xenobiotic-induced hepatic fibrosis (Wolf and Wolfe, 2005), hypohidrotic ectodermal dysplasia (Harris et al, 2014), high-fat diet (HFD)-induced diabetic nephropathy (Ichimura et al, 2013), and chronic mycobacterial infection (tuberculosis; Mosi et al, 2012), to name just a few.

1.1. One Point is a Datum, Two Points Provide Data

The history of science documents that complex problems are often best addressed using a comparative approach. Comparing two related species allows one to address general genetic principles in eukaryotes and find related physiological patterns among organisms that have evolved alternative lifestyles and under very different physical or environmental conditions. For example, in yeast, the *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* systems have demonstrated that species-specific differences in many biological features coupled with their phylogenetic distance make them both valuable in comparative approaches to complex questions (Russell and Nurse, 1986; Mata and Nurse, 1998). It has also been shown that organisms initially selected to be the "best models" for laboratory growth and ease of use may later be shown to represent evolutionary outliers. Although zebrafish represents an extremely valuable model for developmental biology, the recent advent of large-scale genomics has demonstrated this model does not exhibit the extent of conserved synteny present in medaka and other fish models (Wittbrodt et al., 2002; Amores et al, 2014). There is no doubt the zebrafish model is scientifically impacting, but added data from comparative models such as medaka will allow the findings in zebrafish to be vetted and the data to provide a deeper understating of human disease.

Among Teleost fishes, *Fundulus* and *Xiphophorus* are members of the order Cyprinodontiformes and medaka is a member of the sister order Beloniformes, while zebrafish (*Danio*) are members of the order Cypriniformes. Among these four species, each representing varied experimental models of biomedical importance, *Xiphophorus* (live bearing) and medaka (egg laying) are the two closest relatives (divergence about 100 million years ago). The order Cypriniformes, which includes zebrafish, blind cavefish, and goldfish, and the Cyprinodontiformes are estimated to have diverged about 300 million years ago (Mya), representing a genetic distance similar to that estimated for human and chicken (≈310 Mya) (Kumar and Hedges, 1998; Postlethwait et al., 2000; Steinke et al, 2006).

The evolutionary distance between these various biomedical models provides extreme strength to comparative approaches where experimental results from side-by-side analyses using two or more models either strengthen the findings, if the models agree, or provides insight into alternative mechanisms and thus aides our understating, if they do not agree. The parallel biological and experimental attributes of both medaka and zebrafish (clear embryos, well-understood development, ample mutants, capability to perform mutant screens, CRISPR/Cas9 KO collections, genome resources, etc.) allow techniques and methods developed for one system to be easily transferred to the other (Wittbrodt et al, 2002; Furutani-Seiki and Wittbrodt, 2004). Thus, using two models to study the same variables provide scientists with an extremely powerful comparative experimental tandem that can be applied to complex problems such as the etiology and progression of human disease.

1.2. Medaka resources in the USA

Until a few years ago, the University of Georgia maintained a medaka resource center under the oversight of Dr. Richard Winn, who had created lambda rescue medaka fish models for mutagenesis research (i.e., analogous to the Big Blue Mouse) (Hill et al., 1999). This facility provided healthy fish to researchers for a modest cost and had begun to collect mutant medaka strains that researchers could request. Unfortunately, the Georgia facility has closed, and the medaka lines it had maintained have been scattered into two or three independent laboratories. Currently, US researchers have no reliable source of healthy and standardized medaka fish for research from any established center or laboratory.

Participants in the workshop representing the international medaka community have already transitioned through similar issues in Europe or Asia and thus could lend their experiences to the US-oriented group (see Appendix 2). The workshop focus was to first address the issues experienced by medaka scientists in the US and then to engage the group in directed discussion to propose mechanisms that may best address the issues. Below we present a summary of the presentations, discussions, and recommendations forwarded by the workshop attendees.

2. Summary of presentations and discussion

2.1. Session 1: The Medaka Model and Human Disease. Dr. Tomoko Obara, Moderator (University of Oklahoma Health Sciences Center)

In the first session, scientists presented data on five established aquatic models highlighting the use of medaka in human disease research and the novel findings acquired using this model system.

Dr. Tomoko Obara led the session with a short history of the medaka model using materials provided by Dr. Aki Shima. Dr. Shima's presentation documented research stretching from 1921 and included the 40+ years he has utilized this model in radiation exposure research and other studies. The early details of medaka development, remarkable genetic polymorphisms that exist among various medaka populations, and results from experimental studies clearly showing the effect of temperature on development, toxicity, and radiation sensitivity were presented.

Dr. Manfred Schartl has developed a melanoma model in medaka by producing a transgenic model carrying the dominant *Xmrk* oncogene from *Xiphophorus*. This driver oncogene gene, when expressed under control of the *Mitf* promoter, leads to the development of melanoma (100% penetrance) as early as 2–3 weeks post-hatch and is currently being developed for use in drug screening to identify small molecules that may inhibit melanoma progression. He presented RNASeq results showing that global transcription in this medaka melanoma model