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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.

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Keywords

Medaka; *Oryzias latipes*; ricefish; model organism; biomedical research; translational model; zebrafish

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*; Scharf 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, Ichimura et al., 2013) than zebrafish. Medaka possesses a small genome (700 Mb, less than half the size of the 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 US. 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 et al, 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.

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.

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.

Summary of Presentations and Discussion

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 Scharfl 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 were in excellent agreement with gene expression results determined from human tumor samples.

Dr. Scharfl also presented a newly developing medaka resource at the Karlsruhe Institute of Technology (KIT; Germany) forwarded by Dr. Joachim Wittbrodt (mentioned above). KIT is producing a population genetics resource by inbreeding 150 medaka lines derived from widely variant populations. These lines are now 9 generations inbred and currently 24 of the genomes have been sequenced at 9× coverage for each line. Each of the medaka lines are being phenotyped for various differences in adult morphology, behavioral traits, cancer susceptibility, innate immunity, general metabolism, and many other quantifiable phenotypes. Also, each line will have a transgene receiver inserted at precisely the same location in the genome of all lines. This receiver locus will allow one to produce transgenic fish carrying any driver gene of interest. Once this system is operational, visiting scientists may assess the effects of these many variable genetic backgrounds on any driver gene (e.g., oncogene, transcription factor, etc.) and assess the effects of any driver gene on gene-environment interactions and complex or multigenic traits. Such medaka resources promise to provide novel insight into genetic modifiers and will produce experimentation to address novel biomedical questions (Spivakov et al, 2014).

Dr. Christoph Winkler presented results from his work on medaka models of osteoporosis. He showed that there are interesting differences between zebrafish and medaka upon creation of CRISPR/Cas9 knockout (KO) of the same orthologous *osx* (*osterix*) gene. Whereas zebrafish KOs do not show an early bone phenotype, the medaka KOs present severe defects in bone formation. The medaka *osx* KO has developed into an osteoporosis model by live imaging of osteoclast-osteoblast function during larval and adult stages. He also presented an adult over-ossification (osteopetrosis) model, where osteoclast deficiency leads to severe bone defects thus illustrating the necessity of bone remodeling in fish. For bone deposition studies, the zebrafish and mouse have proven to be problematic models, whereas specific attributes of medaka have produced an informative system that will forward our understanding of bone disease.

Dr. Dave Hinton presented data from his extensive experience in both descriptive morphology and disease pathology using the medaka model in environmental toxicity studies. He compared medaka pathology with that of many other small fishes and informed the workshop attendees that notable species-specific differences may be observed. His pioneering work served to help establish medaka as a mainstream toxicology model in the

US over the past 30 years. Seminal studies and ongoing work showing medaka hepatic fibrosis from various chemical exposures, including discharge from coal fired power plants, have utilized medaka as a biological indicator species in long-term toxicity testing regimen. Established pathological differences in medaka liver tumorigenesis associated with the embryonic timing at exposure were shown and discussed. In addition, pathological differences in, gender specific tumor responses, effects of tumor promoting agents, and the potential of tumor metastasis were presented. Overall, medaka has been shown to meet the rigid standards established by the US National Toxicology Program for carcinogenicity testing, and is used as a aquatic toxicology model by regulatory agencies such as the US Environmental Protection Agency. However, the well-described differences among these fishes, and between fishes and other vertebrates, will provide many opportunities to advance our fundamental understanding in environmental toxicology.

To complete the first session, Dr. Seth Kullman showed results from the extensive analyses of his laboratory using the medaka model to study TCDD induced dysmorphogenesis. In particular, he showed TCCD effects on skeletal development. Bone deposition is severely dysregulated upon developmental activation of the AhR pathways due to exposure to TCDD, even at parts per trillion levels. These effects agree well with alterations in cell proliferation and cell migration and were supported with direct measures of specific gene expression in bone and cartilage. In addition, Dr. Kullman presented results from studies of the vitamin D receptor responses as a developing model of neurodegenerative disease in humans. Both zebrafish and medaka have similar vitamin D receptor gene duplicates but the evolutionary divergence between them makes the two fishes differentially susceptible to assess receptor agonists/antagonists, and thus simultaneous comparison of the two models becomes informative and essential. Overall, the results presented indicate medaka may serve as an excellent model to assess gene-environment-disease interactions.

Session 2: Comparative Medaka and Zebrafish Models. Dr. Dave Hinton, moderator (Duke University)

In the second session, four investigators presented their experimental results regarding the value and use of medaka and additional aquatic models, in comparative studies.

Dr. Mathew Harris led the second session detailing his work showing how medaka and zebrafish are unique and valuable as comparative models. Both zebrafish and medaka serve as excellent comparators as similar experiments can be run on both in a common facility. This experimental ability permits elegant analysis of the diversity of gene function in development and physiology. For example, exposure to the drug FK506, a calcineurin binding factor, induced increased growth in clipped zebrafish fins, but had no effect on fin growth rate in medaka. Further, a gene knockout of the deacetylase *sirt1* in zebrafish causes erratic or spiraling swimming patterns, but the same KO gene in medaka has no detectable phenotype even though the function of this gene is highly conserved from yeast to humans. There are many other such differences and similarities the Harris laboratory finds in comparative genetic studies in both zebrafish and medaka. The value of paired studies is to enforce findings of gene function by agreement, or to illuminate the problem at hand by

revealing differences in genetic response. This makes paired studies highly informative, and essential for understanding the etiology of disease.

Dr. Tomoko Obara presented results from her studies showing that medaka provides a translational model for diabetic nephropathy, where currently no rodent animal model has met the criteria established by the Animal Models of Diabetic Complications Consortium (AMDCC). To develop this model, both zebrafish and medaka were fed a high fat diet (HFD), but only medaka expressed the elevated blood glucose levels, enlarged glomeruli, and glomerular capillary dilation that are characteristic of diabetic nephropathy. Zebrafish did not show elevated blood glucose (Ichimura et al., 2013). These same diagnostic criteria for diabetic nephropathy were confirmed in a medaka mutant for the *neprilysin* gene, and this mirrors down regulation of *NEPRILYSIN* in human diabetic nephropathy. Further, the condition could be reversed in HFD fed medaka with the treatment of an inhibitor of the Angiotension II receptor, as one may predict from the human physiological condition.

Other results from the Obara laboratory include producing a new model for generation of nephron structures in medaka using adult mouse kidney cell transplants. Such diabetic nephropathy and nephron development models have not, as of yet, been produced in zebrafish despite considerable effort and comparative experimentation.

Dr. Shosaku Kashiwada has been using medaka in toxicological studies of nanoparticles, a new and widely used commodity with a paucity of knowledge of health effects. He uses the “see-through” medaka mutant to allow direct visualization of nanoparticle uptake and compartmentalization *in situ*. He presented results from these studies showing effect of nanoparticles exposure on glycosylation that, in turn, affected normal morphogenesis. These novel glycobiology studies were corroborated by microarray and qRT-PCR analyses, confirming dysregulation of glycosylating enzymes with exposure to nanoparticles.

Dr. Don Ennis has been using medaka to study infection by *Mycobacterium marinum* (Mm), one of the closest relatives of the tuberculosis-causing human pathogen, *Mycobacterium tuberculosis* (Mtb). Because Mm causes a TB-like disease in fish it has been employed as a cost-effective surrogate model for human tuberculosis (TB). This pioneering work compared “fish TB” in both medaka and zebrafish serving as companion models for both acute and chronic human TB. That is, zebrafish are hypersensitive to infection resulting in global inflammation of the viscera, producing an acute disease and leading to high mortality. In contrast, several other fish models like goldfish, tilapia, and medaka respond very differently to mycobacterial infections. Once infected, they produce a chronic disease more similar to the two billion human TB cases worldwide. Dr. Ennis’ group has employed mosquito larvae, which have fed on Mm as a natural vessel, to deliver infectious oral doses to medaka. It was discovered that passage of the larvae through the digestive tract serves to activate Mm virulence genes and substantially augment bacterial infectivity (e.g., 100–1,000-fold). In a matter of weeks following ingestion, the bacteria were found to not only cross the epithelia of the gastrointestinal tract but will colonize organs such as the spleen, kidney, and liver. In human TB patients, infected carriers may remain in a chronic infection state for decades, but each year a small subset (~1%) will suddenly transition to an acute disease phase resulting in severe incapacitation and often death (2 million annually). The use of the medaka to model

the chronic TB, and the zebrafish to model acute infections, again underscores the power of using both models in a comparative manner to advance a better understanding of this devastating human disease. It has been well documented that small fish models can be utilized in high-throughput screening to identify new drugs for treatment of human diseases, and these companion fish models offer platforms for the identification of anti-TB drugs.

Session 3: Enhancement of Impact for Medaka as a Comparative Research Model to Investigate of Human Disease. Dr. Ron Walter, moderator (Texas State University)

Summary of Discussions—Throughout the workshop and presentations, participants engaged in discussions about the usefulness of developing medaka as a standard aquatic model and how to accomplish this goal. The final workshop session was devoted to an open round table discussion aimed at defining specific resource needs the medaka community envision as essential to their ability to perform higher impact studies, and what hurdles prevent the medaka model from being more widely adopted for comparative studies. These discussions centered around five central areas; (1) establishment and characterization of standard medaka lines, (2) genomic resource development, (3) a digital resource for genetic information, provision of medaka protocols, SOPs and IACUC drafts, (4) medaka cell lines, and (5) medaka pathology and diagnoses.

(1) Establishment and characterization of standard medaka lines: One of the first needs discussed was the adoption and availability of standard medaka reference lines. Discussion of several medaka lines that each of the represented laboratories utilize as a reference determined that all were offshoots of the Cab line initially sold by Carolina Biological Supply Inc. many years ago. Several stocks from this Cab line are used by current laboratories both nationally and internationally, but experimental results indicate these lines may be somewhat variable. The representatives from Duke and North Carolina State Universities have their own Cab stock that they have given out on occasion in the US, while the European line was most likely also derived from a Cab line Dr. Joachim Wittbrodt inbred many years ago, and gave out to several laboratories. A second reference line is the Hdr line that was utilized as a source of the medaka genome sequence and assembly. The National Institute of Basic Biology at the University of Tokyo (NIB; under the direction of Dr. Kiyoshi Naruse) may provide Cab (derived many years ago from the Wittbrodt's line) or Hdr, as well as many other different medaka strains and species. The NIB in Japan, has been and remains cooperative to international researchers, but post 911 shipping of fishes internationally has become quite difficult. Further, the laboratories represented are often asked to provide medaka to external or internal research groups, but they have no dedicated resources for this effort. There is a fundamental need for the community to decide on a few standard reference line(s), to characterize these reference line(s), and then to establish a mechanism to rear and distribute them in the US.

(2) Genomic resources: The medaka genome was one of the earliest fish genomes sequenced. However, assessment of the current medaka reference (vs. HdrR) identifies about 170 Mb of missing sequences among the assembly scaffolds. Also, over 10% of the annotated gene models are considered incomplete. The Cab and Hdr lines were recommended to have their genomes *de novo* sequenced and assembled using contemporary

technologies leading to production of a more informative genomic resource. In addition, discussions centered on a need to provide both SNP calls and RNA transcriptome comparative data for the most commonly used medaka reference lines.

Overall, the discussants considered establishment of standard lines, provision of contemporary genome resources and documentation of genetic diversity between the lines as major first steps forward in promoting the medaka model.

(3) Digital medaka resources: The discussants considered establishment of a medaka genomic digital data repository a high priority. This digital resource could potentially host a genome browser, offer “live genome” mechanisms for editing, provide a repository of mutant screen and drug screening information, KOs and mutant line documentation, and most importantly, coordination for the alignment of nomenclature between medaka and zebrafish as important issues.

In addition, there was agreement that one big hurdle keeping new investigators from adopting medaka in their studies was the lack of knowledge about medaka husbandry; the standard protocols for strain maintenance and their use in a research setting. Although this is similar to zebrafish, medaka do require some modification in the care regimen utilized in animal facilities. It is thus necessary to establish a web-interfaced location where information (SOPs, and IACUC protocols) is freely available for users.

(4) Medaka cell lines: A needed area of medaka resource improvement involves establishment of a distribution source of medaka cell lines. Cell lines, mostly fibroblasts, are already available from various laboratories and some of these have been used for many years. However protocols for their use and the distribution of these lines are an issue. New non-fibroblast cell lines and medaka stem cells have been developed and offer many new inroads into research, if they can be adequately handled and distributed.

(5) Medaka pathology and diagnosis: Given the use of medaka in toxicological assessment, there exists unique and ample historical data regarding medaka anatomy and pathology. A valuable asset to medaka is the presence of several active pathologists that are very familiar with this model. Pathologists with small fish expertise are rare and the availability of active medaka pathologists may allow medaka to become a central resource for small fish pathology in both a live and digital sense. Digital libraries of pathological examples may be made available for researchers at remote locations with pathology training or facilities. The possibility of establishing a pathology resource laboratory where external scientists may send samples for diagnosis and consultation would propel this model into new areas and considerably strengthen the translational reach of data derived from using the medaka model.

Workshop Recommendations—Given the documented capabilities of medaka to uniquely, or in comparative studies with zebrafish, increase our knowledge and understanding of human disease the following three recommendations are discussed by the workshop attendees for consideration:

Recommendation (1): To establish a Medaka Resource Center to serve the US scientific community by spearheading the establishment, genetic characterization, and distribution of standardized medaka lines. Additionally, the center will assume oversight in development of medaka genomic resources and produce a digital repository of medaka information with appropriate web-based access to medaka lines, mutant lines, mutant screens, genetic information, and other data; as well as serving as a source of medaka SOPs and protocols that will enhance the likelihood that new investigators will utilize the medaka model. The established medaka center will also serve as the point of outreach and interaction with zebrafish resources, here and abroad, to ensure consistent nomenclature and to provide maximal information content for data derived from medaka and zebrafish comparative studies.

Mechanism: The above recommendation calls for re-establishment of a Medaka Resource Center in the US. The NIB center in Japan pledged strong support for such an endeavor and the proposed center may rely on the experience and lines at NIB as it develops.

There was considerable discussion on the mechanisms for initiating a medaka resource center as either a centralized facility or as a distribution and interactive center where each directive may be assigned to different entities at different locations. The workshop attendees favored a centralized facility for medaka fish lines, mutants, and genomics data to start the process, with other components that may need to be developed over time perhaps distributed to satellite sites, but coordinated from the central medaka resource that maintains and distributes medaka fishes to the community.

Recommendation (2): It is recommended that a resource for creation, characterization, and distribution of medaka cells lines be established. The first priority for medaka cells lines would be for agreed upon reference lines. It is important that this resource center have the ability to assist external investigators in the use of new non-fibroblast stem cell lines..

Mechanism: Although many laboratories utilize medaka fibroblast lines, establishment of nonfibroblast lines, and the development of stem cell resources requires specialized expertise. The specialized talents for cell lines and non-fibroblast stem cells is distinct from that needed to provide medaka husbandry, distribution, and oversight of genomic resources; and thus is not necessarily a part of the above recommended medaka resource center. To address this recommendation will require commitment from a select group of scientists having specialized expertise and who are willing to provide this as a service to the community.

Recommendation (3): It is recommended that a pathological resource be developed to provide digital resources of historical pathological diagnoses references and who may provide assistance to researchers that produce and use medaka in experimentation.

Mechanism: As discussed for recommendation 2 (above), realization of a medaka pathology resource will fall on a small group of scientists having specialized expertise. However, the medaka model is fortunate that such a group and pathological history do exist and the

enlistment of these professionals in assisting medaka research studies places this experimental model on firm footing to be optimally translational to human disease.

Concluding remarks

Based on the above three recommendations there is a clear path forward for medaka researchers in the USA to begin to develop the needed infrastructure. Individually, or in cohorts, each of these recommendations may be developed into a proposal for funding to be submitted to appropriate federal agencies. Proposals that address these recommendations will have the support of the medaka research community. Alternatively, each of the recommendations may be utilized as a focal point to meet and develop a “white paper” that may be forwarded to federal agencies for consideration of focused support. By either mechanism, or others, fulfillment of these recommendations require a commitment of service to the research community by those capable of establishing the intended infrastructure improvements. It is our collective hope that leaders will arise from the medaka research community and they will accept the challenges to the betterment of all.

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Literature Cited

- Aida T. On the Inheritance of color in a freshwater fish, *Aplocheilichthys latipes* Temmick and Schlegel, with special reference to sex-linked inheritance. *Genetics*. 1921; 6:554–573. [PubMed: 17245975]
- Amores A, Catchen C, Nanda I, Warren W, Walter RB, Schartl M, Postlethwait JH. A Rad-tag genetic map for the platyfish (*Xiphophorus maculatus*) reveals mechanisms of karyotype evolution among teleost fish. *Genetics*. 2014; 197(2):625–641. [PubMed: 24700104]
- Furutani-Seiki M, Wittbrodt J. Medaka and zebrafish and evolutionary twin study. *Mech. Develop.* 2004; 121(7–8):629–637.
- Harris MP, Henke K, Hawkins MB, Witten PE. Fish is Fish: the use of experimental model species to reveal causes of skeletal diversity in evolution and disease. *J. Appl. Ichth.* 2014; 30:616–629.
- Hill KA, Nishino H, Buettner VL, Halangoda A, Li W, Sommer SS. The Big Blue(R) transgenic mouse mutation detection assay: the mutation pattern of sector mutant plaques. *Mutat. Res.* 1999; 425(1):47–54. 10. [PubMed: 10082915]
- Ichimura K, Kawashima Y, Nakamura T, Powell R, Hidoh Y, Shuji T, Sakaida I, Kadera Y, Tsuji T, Ma J-X, Saki T, Matsumoto H, Obara T. Medaka fish, *Oryzias latipes* as a model for human obesity-related glomerulopathy. *Biochem. Biophys. Res. Com.* 2013; 431:712–717.
- Ichimura K, Bubenshchikova E, Powell R, Fukuyo Y, Nakamura T, Tran U, Oda S, Tanaka M, Wessely O, Kurihara H, Sakai T, Obara T. A comparative analysis of glomerulus development in the pronephros of medaka and zebrafish. *PLoS One*. 2012; 7(9):e45286. [PubMed: 23028906]
- Kasahara M, Naruse K, Sasaki S, Nakatani Y, Qu W, Ahsan B, Yamada T, Nagayasu Y, Doi K, Kasai Y, Jindo T, Kobayashi D, Shimada A, Toyoda A, Kuroki Y, Fujiyama A, Sasaki T, Shimizu A, Asakawa S, Shimizu N, Hashimoto S, Yang J, Lee Y, Matsushima K, Sugano S, Sakaizumi M, Narita T, Ohishi K, Haga S, Ohta F, Nomoto H, Nogata K, Morishita T, Endo T, Shin-I T, Takeda

- H, Morishita S, Kohara Y. The medaka draft genome and insights into vertebrate genome evolution. *Nature*. 2007; 447:714–719. [PubMed: 17554307]
- Kirchmaier S, Naruse K, Wittbrodt J, Loosli F. The genomics and genetic toolbox of the teleost medaka (*Oryzias latipes*). *Genetics*. 2015; 199:905–918. [PubMed: 25855651]
- Kitambi SS, Malicki JJ. Spatiotemporal features of neurogenesis in the retina of medaka, *Oryzias latipes*. *Devolp. Dyn*. 2008; 237(12):3870–3881.
- Kumar S, Hedges SB. A molecular timescale for vertebrate evolution. *Nature*. 1998; 392:917–920. [PubMed: 9582070]
- Mata J, Nurse P. Discovering the poles in yeast. *Trends Cell Biol*. 1998; 8(4):163–167. 8. [PubMed: 9695831]
- Mosi L, Mutoji NK, Basile FA, Donnell R, Jackson KL, et al. *Mycobacterium ulcerans* causes minimal pathogenesis and colonization in medaka (*Oryzias latipes*): an experimental fish model of disease transmission. *Microbes Infect*. 2012; 14:719–729. [PubMed: 22465732]
- Naruse K, Hori H, Shimizu N, Kohara Y, Takeda H. Medaka genomics: a bridge between mutant phenotype and gene function. *Mechan. Dev. (Review)*. 2004; 121(7–8):619–628.
- Postlethwait JH, Woods IG, Ngo-Hazelett P, Yan YL, Kelly PD, Chu F, Huang H, Hill-Force A, Talbot WS. Zebrafish comparative genomics and the origins of vertebrate chromosomes. *Genome Res*. 2000; 10(12):1890–18902. [PubMed: 11116085]
- Russell P, Nurse P. *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*: a look at yeasts divided. *Cell*. 1986; 45(6):781–782. [PubMed: 3518949]
- Schartl M, Wilde B, Laisney JA, Taniguchi Y, Takeda S, Meierjohann S. A mutated EGFR is sufficient to induce malignant melanoma with genetic background-dependent histopathologies. *J. Invest. Dermatol*. 2010; 130:249–258. [PubMed: 19609310]
- Spivakov M, Auer TO, Peravali R, Dunham I, Dolle D, Fujiyama A, Toyeda A, Aizu T, Minakuchi Y, Loosli F, Naruse K, Birney E, Wittbrodt J. Genomic and Phenotypic Characterization of a Wild Medaka Population: Towards the Establishment of an Isogenic Population Genetic Resource in Fish. *G3:Genes Genomes Genetics*. 2014; 4(3):433–445. [PubMed: 24408034]
- Steinke D, Salzburger W, Meyer A. Novel Relationships Among Ten Fish Model Species Revealed Based on a Phylogenomic Analysis Using ESTs. *J. Mol. Evol*. 2006; 62(6):772–784. [PubMed: 16752215]
- To TT, Witten PE, Renn J, Bhattacharya D, Huysseune A, Winkler C. Rankl-induced osteoclastogenesis leads to loss of mineralization in a medaka osteoporosis model. *Development*. 2012; 139:141–150. [PubMed: 22096076]
- Wolf JC, Wolfe MJ. A brief overview of nonneoplastic hepatic toxicity in fish. *Toxicol Pathol*. 2005; 33:75–85. [PubMed: 15805058]
- Wakamatsu Y, Pristiyazhnyuk S, Kinoshita M, Tanaka M, Ozato K. The see-through medaka: a fish model that is transparent throughout life. *Proc. Natl. Acad. Sci. USA*. 2001; 98:10046–10050. [PubMed: 11526229]
- Wittbrodt J, Shima A, Schartl M. Medaka - A model organism from the far East. *Nat. Rev. Genet*. 2002; 3:53–64. [PubMed: 11823791]

Appendix 1: Workshop Agenda

The Medaka Model for Comparative Assessment of Human Disease Mechanisms Hilton Inn at Austin-Bergstrom International Airport December 18, 2014

1:00 – 1:15 pm	Welcoming Remarks Miguel Contreras, Health Science Administrator, DCM
1:15 – 1:30 pm	Opening Remarks and Workshop Goals: Medaka Resources and Impacts Ron Walter, Texas State University

1:25 – 3:05 pm	The Medaka Model and Human Disease (Tomoko Obara, Moderator) Short talks on capabilities of the medaka to model human disease Aki Shima (by Tomoko Obara), Manfred Scharl, Christoph Winkler, Hinton, Seth Kullman (15 min. + 5 min. discussion for each talk)
3:05 – 3:20 pm	Break
3:20 – 5:00 pm	One point is datum, two make data: Comparative Medaka and Zebrafish Models (Dave Hinton, Moderator) Short talks on comparative utility of medaka to model human disease Matthew Harris, Tomoko Obara, Shosaku Kashiwada, Don Ennis (15 min. + 5 min. discussion for each talk)
5:00 – 7:00 pm	Break for Dinner
7:00 – 8:30 pm	Recommendations on Mechanisms to Enhance the Impact of Medaka as a Comparative Research Model for Investigation of Human Disease (Ron Walter, Moderator)

Appendix 2: List of Workshop Participants

NIH Attendees

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In abstentia

Minoru Tanaka, Ph.D., *In abstentia*, (University of Tokyo, Japan), Tomoko Obara presents

Akihiro Shima, Ph.D., *In abstentia*, (University of Tokyo, Japan), Tomoko Obara presents

Joachim Wittbrodt, Ph.D., *In abstentia* (University of Heidelberg), Manfred Scharlt presents