The Medaka Model for Comparative Assessment of Human Disease Mechanisms

Austin, TX

December 18th, 2014

Workshop Report

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A. Executive Summary

The Japanese medaka (*Oryzias latipes*) has a scientific history dating back to 1921 and, with the exception of zebrafish, is the most studied teleost experimental model employed in biomedical research. Medaka are utilized worldwide and have available substantial resources, including a fully sequenced genome, high resolution genetic maps, inbred lines, hundreds of mutants, independently derived wild stocks with substantial phenotypic variation, transgenic capabilities, and many more. Unfortunately, many of these resources are not directly available to American biomedical researchers because they are located outside the United States.

Medaka provide comparative advantages with the use of zebrafish as biomedical models. For example, mutants in orthologous genes in zebrafish and medaka often have subtle, and sometimes substantial, differences in phenotype and thus reliance on one model may lead to erroneous translational generalizations about the human condition being studied. Medaka normally occur in Japan, where animals are available in the wild and possess naturally occurring variation that is accessible and 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 having several different genetic backgrounds that all carry the same melanoma driver gene from *Xiphophorus* (i.e., *Xmrk2*). In this case, the same driver gene construct led to development of different tumor types in each of the three varied medaka genetic backgrounds (melanoma in HB32C, exophytic xanthoerythrophoroma in *Carbio,* and retinoblastoma in the *i-3* genetic background). This natural variation among medaka is being exploited at the Karlsruhe Institute of Technology (Germany), where a population genetics resource is being produced by inbreeding 150 medaka lines that are derived from widely variant wild populations. Genome sequences will be produced for each line and each line will be phenotyped for many and varied differences (e.g., morphology, behavior, disease susceptibility, immunity, etc.). 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.). There is no resource such as this available for the zebrafish or any other aquatic model.

Medaka, like zebrafish, are oviparous and have a clear chorion allowing easy visualization of all stages of early development, from the single cell to the free swimming hatchling. “See through” medaka lines lack all pigment and thus allow fluorescent visualization of gene expression within the living animal at any developmental stage. From an evolutionary viewpoint, medaka are much more closely related to other commonly utilized experimental fish models (i.e., *Xiphophorus*, Stickleback, *Fugu*, *Fundulus*, etc.) than are zebrafish. Medaka possess 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. 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.

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 United States**.** Presentations at the workshop documented medaka as a valuable comparative model along with the zebrafish; however, ***many newly developed medaka models are able to provide more directly translational understating of the human condition for diseases such as osteoporosis, xenobiotic induced hepatic fibrosis, hypohidrotic ectodermal dysplasia, diabetic nephropathy, and chronic mycobacterial infection (tuberculosis), to name just a few.***

B. Goals and Objectives

Results of recent studies showing the utility of medaka to model human disease states were presented at the 7th Aquatic Models of Human Disease Conference that was held outside of Austin, TX on December 13-18, 2014. This conference brought together many of the highest regarded national and international medaka scientists. To take advantage of this opportunity, the most established and experienced medaka researchers were invited to stay an extra day and take part in a workshop entitled "*The Medaka Model for Comparative Assessment of Human Disease Mechanisms"*.

The central purpose of this workshop was to assess current use and to 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 scientists in the United States to perform experiments leading to impacting experimental results directly relevant in human disease research. Consistent with this central purpose, the workshop was divided into three sessions, two sessions of presentations by invited speakers having expertise and experience in the session topics, and the third one for round table discussions. The workshop hosted 20 scientific participants (Appendix 2) and of these, nine scientists presented formal talks.

This document is a summary report stemming from workshop presentations, subsequent round table discussions, and recommendations from this group that represent views of the overall medaka research community.

C. Introduction to the Medaka Model

The Medaka Model.

Medaka are small, egg-laying, freshwater, bony fish that are native to Japan, Korea, and China. Among Teleost fishes, *Fundulus*, and *Xiphophorus* are members of the order Cyprinodontiformes and medaka are members 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 (diverged 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, representing a genetic distance similar to that estimated between human and chicken (about 310 million years ago).

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 aide our understanding, 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. Thus, ***the models together 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.***

Although medaka are not as commonly used as zebrafish in the US, this aquatic species has several unique advantages; a small well conserved genome, the existence of over 20 species spread throughout Asia that exhibit a high degree of genetic variability, highly fertile and inbred strains, toleration of a wide temperature range (4 - 40 ºC) during embryonic development, and species

adapted to both fresh water and saltwater environments.

Due to 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 (about 60 million years diverged) in contrast to zebrafish. However, given the early sequencing of the medaka genome, the medaka assembly could be vastly and quickly improved by application of newer technologies. The National Institute of Basic Biology at the University of Tokyo maintains a very impressive medaka resource center for both medaka fish and experimental resources related to research use of medaka. However, due to post 911 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.

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. Comparison of 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 perhaps under very different physical or biological conditions. For example, in yeast, the *Saccharomyces cerevisia*e 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. 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. There is no doubt the zebrafish model is impacting, but added data from comparative models such as medaka, will allow the findings in zebrafish to be vetted and the data to be more translatable to the human condition.

Medaka Resources in the Unites States.

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). 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, researchers in the United States have no local and 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 Unites States-oriented group (see Appendix 2). The focus of the workshops was to engage participants in directed discussion to propose mechanisms that may best address the issues experienced by medaka scientists in the United States. Below is presented a summary of the presentations, discussions, and recommendations advanced by the workshop attendees.

D. Summary of Presentations and Discussion

<u>Session 1</u>: 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 (University of Tokyo, Japan). 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 between various medaka populations, and results from experimental studies clearly showing the effect of temperature on development, toxicity, and radiation sensitivity were presented.

Professor Manfred Schartl (Department Physiologische Chemie, Biozentrum, Germany) has developed a melanoma model in medaka by producing a transgenic model carrying the dominant *Xmrk* oncogene from *Xiphophorus*. This driver oncogene gene, when regulated by 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.

Professor Schartl also presented a newly developing medaka resource at the Karlsruhe Institute of Technology (KIT; Germany) developed by Dr. Joachim Wittbrodt (as mentioned in the Executive Summary). 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 9x 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 production of 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 experiments to address novel biomedical questions.

Dr. Christoph Winkler (National University of Singapore, Singapore) 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 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 the understanding of bone disease.

Professor Dave Hinton (Duke University) 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 highlighting

that notable species-specific difference may be observed. His pioneering work helped to establish medaka as a mainstream toxicology model in the United States 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 regimens. Established pathological differences in medaka liver tumorigenesis associated with the embryonic timing at exposure were shown and discussed. In addition, pathological differences, sex specific tumor responses, effects of tumor promoting agents, and the potential of tumor metastasis were presented. Overall, medaka have been shown to meet the rigid standards established by the United States National Toxicology Program for carcinogenicity testing, and are used as an aquatic toxicology model by regulatory agencies such as the United States Environmental Protection Agency. However, the well-described differences among these fishes, and between fishes and other vertebrates, will provide many opportunities to move forward fundamental understanding in environmental toxicology.

To complete the first session, Dr. Seth Kullman (North Carolina State University) showed results from the extensive analyses of his laboratory using the medaka model to study 2,3,7,8-Tetrachlorodibenzodioxin (TCDD) induced dysmorphogenesis. In particular, he showed TCDD effects on skeletal development. Bone deposition is severely dysregulated upon developmental activation of the AhR pathways due to exposure to TCDD, even at part per trillion levels. These effects agree well with alterations in cell proliferation and cell migration and where supported with direct measures of specific gene expression in bone and cartilage. In addition, Dr. Kullman presented results from studies of 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 that 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 outstanding experts presented their experimental results regarding the value and use of medaka and additional aquatic models in comparative studies.

Dr. Mathew Harris (Harvard Medical School) 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. Such paired studies should be encouraged both in basic and applied applications.

Dr. Tomoko Obara (University of Oklahoma Health Sciences Center) presented results from her studies showing that medaka provide 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. 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. 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 disease. Further, the condition could be reversed in HFD fed medaka with the treatment of an inhibitor of the angiotension II receptor, as would be predicted from the human 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 (Toyo University, Japan) 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 proper morphogenesis. These novel glycobiology studies were corroborated by microarray and qRT-PCR analyses, confirming dysregulation of glycosylating enzymes with exposure to nanoparticles.

Professor Don Ennis (University of Louisiana) 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 colonized 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 forward 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 that these companion fish models offer platforms for identification of anti-TB drugs.

<u>Session 3</u>: Recommendations to Enhance Impact of Medaka as a Comparative Research Model for Investigation of Human Disease. Dr. Ron Walter, moderator (Texas State University).

Throughout the workshop and presentations, participants engaged in discussions about the usefulness in developing medaka as a standard aquatic model and how to accomplish this goal, highlighting the need of a *Resource Center*. The final workshop session was devoted to an open round table discussion aimed at defining <u>additional</u> specific resource needs that the medaka community sees 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 diagnosis.

(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 lines that each of the laboratories represented use as a reference line determined that many of these were offshoots of the CAB line initially sold by Carolina Biological Supply Inc. many years ago, but have since been discontinued. A second reference line is the Hdr line that was utilized as a source of the medaka genome sequence and assembly. Several stocks from the 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 Caroline State Universities have their own CAB stock that they have given out on occasion in the United States, 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. 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. Closure of the medaka resource center at the University of Georgia has resulted in no single source for standardized lines in the US that allow researchers, experienced and new, to obtain fish representing the same genetic heritage. Further, the laboratories represented at the workshop 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 standard reference line(s), to work to characterize these reference line(s), and then to establish a mechanism to rear and distribute them in the United States.

(2) Genomic Resource Development

The medaka genome was one of the earliest genomes sequenced. However, assessment of the current medaka reference (vs. Hdr reference) identifies about 170 Mb of missing sequence among the assembly scaffolds. Also, over 10% of the annotated gene models are considered incomplete. The CAB and Hdr reference lines have been forwarded 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 crossing the bridge to establish 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 the establishment of a digital data repository, a centralized resource that: i) host a genome browser offering a "live genome" mechanism for editing; ii) serve as a repository of mutant screen and drug screening information, KOs and mutant line documentation; and most importantly, iii) coordinate the alignment of nomenclature between medaka and zebrafish.

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 experimental protocols) that are needed to rear and use medaka are freely available.

(4) Medaka Cell Lines

A needed area of medaka resource improvement involves establishing 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 could 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.

E. 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 forwarded by the workshop attendees for consideration:

Recommendation (1): *To establish a Medaka Resource Center to serve the scientific community within the United States 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 Resource 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 United States. The NIB center in Japan has pledged strong support for such an endeavor and the “to be” established 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 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 those agreed upon reference lines. It is important that this resource has expertise to produce and assist external investigators with use of new non-fibroblast medaka stem cells.*

Mechanism:

Although many laboratories utilize medaka fibroblast lines, establishment of non-fibroblast lines, and development of stem cell resources requires specialized expertise. The specialized expertise required is separate from that needed to provide medaka husbandry, distribution, and oversight of genomic resources and thus is not necessarily a part of the Medaka Resource Center proposed in Recommendation (1). To address this recommendation will require the 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 that may provide assistance to researchers that produce and use medaka in experimentation.*

Mechanism:

As discussed for recommendation (2), 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.

F. Appendices

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, ORIP/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	**Session 1: 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 Schartl, Christoph Winkler, Dave Hinton, Seth Kullman (15 min. + 5 min. discussion for each talk)
3:05 - 3:20 pm	**Break**
3:20 - 5:00 pm	**Session 2: 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	**Session 3: 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

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In absentia:

Minoru Tanaka, Ph.D., (University of Tokyo, Japan), Dr. Tomoko Obara presents.

Akihiro Shima, Ph.D., (University of Tokyo, Japan), Dr. Tomoko Obara presents.

Joachim Wittbrodt, Ph.D., (University of Heidelberg, Germany), Dr. Manfred Schartl presents.