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Opinion
Developing a Comprehensive Mouse Pathology Program

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Laboratory mice are the animal model of choice for many types of biomedical research, particularly given the logarithmic development of genetically engineered mouse models (6). The growing number of biomedical research scientists using laboratory mice has created a high demand for pathology input and support. This need for pathologists with expertise in mouse pathology comes at a time when there is a general deficit in the supply of veterinary pathologists across the United States and Canada (10). Colleges of veterinary medicine, medical schools, and some private institutions are uniquely positioned to develop comprehensive mouse pathology programs as centers of excellence. These programs could be used to train the personnel needed to rapidly and accurately characterize new mouse models for use in biomedical research and preclinical trials. We discuss the organization, funding, and operation of mouse pathology programs.

Basic Unit

The basic unit for a successful mouse pathology program consists of an experienced senior pathologist, a staff pathologist, and a technician, together with appropriate space and equipment. Access to a high-quality histology laboratory and clinical laboratories that comprise microbiology, virology, clinical chemistry, and hematology is essential. Other technical modalities such as rodent-sized radiographic imaging, electron microscopy, and flow cytometry also should be available (ancillary services). The technician should be well trained in performing necropsies and in preparing tissues and cells for a variety of studies. The staff pathologist should be board certified in anatomic pathology (board certification by the American College of Veterinary Pathologists, ACVP). Additional certification in laboratory animal medicine (American College of Laboratory Animal Medicine, ACLAM) or in clinical pathology (ACVP) is an advantage. The staff pathologist is responsible for supervising daily activities including the work of the technician. The senior pathologist directs and coordinates the overall operation of the program, collaborates with principal investigators in planning their projects and publishing their findings, maintains contact with other experienced pathologists as well as organ- and disease-specific experts, and interacts with an advisory board.

Functioning of the Basic Unit

If the work volume is low, the technician could initially be a part-time individual whose schedule is based largely on the experimental design of ongoing projects. As the volume of work increases, additional technicians and pathologists will be needed to share the workload and provide backup. Regardless of the overall program size, all team members must be committed to creating, updating, and following a schedule for each project.

Necropsies must be performed under Institutional Animal Care and Use Committee (IACUC)-approved protocols using American Veterinary Medical Association (AVMA)-endorsed methods of euthanasia (1, 19, 21). The technician should have access to a variety of fixatives, liquid nitrogen, –80°C freezers, microbiological collection materials, and other supplies to accommodate specific needs of investigators. Tissues are trimmed by the technician and submitted in cassettes to a histology laboratory. The histology laboratory should consistently produce excellent stained sections including those created by using standard hematoxylin and eosin stain, special stains, and immunohistochemical techniques. These are minimal requirements which, ideally, will be supplemented by additional capabilities such as in situ hybridization and laser capture microdissection. The latter is a technique that operationally weds light microscopy to molecular analysis. Small groups of cells in sections of fixed or frozen tissue can be excised for studies of DNA, RNA, or protein (http://dir.nichd.nih.gov/lcm/cm.htm). Training programs and modules for technicians and pathologists are available through the American Association for Laboratory Animal Science (AALAS) and at several research and academic institutions (Table 1).

The pathologists will need double-headed microscopes with real-time teaching and imaging capabilities (analog TV camera and monitor or digital camera with real-time capabilities). Ideally, a four-headed microscope or a microscope projection system should be available for regular review of histologic findings with the project teams and with trainees. Alternatively, it is now possible, using existing technology, to capture and display whole-slide images (e.g., Aperio Scanscope, Aperio Technologies, http://www.aperio.com), which can then be placed online (16) and viewed simultaneously at multiple sites for teleconferencing (5, 16). The pathologists will also need protected time for their own research and for preparation of reports, grant applications, and manuscripts.

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Table 1. Web-based databases providing information on comparative pathology and courses useful when setting up a mouse pathology program

Resource	URL
Comparative Pathology of Human and Mouse Breast Cancer	http://www-p.ucdavis.edu/tgmice/cmpath/abstract.htm
General Mouse Pathology	http://www.pathbase.net
Pathology of Mouse Models for Human Diseases	http://www.jax.org/courses/index.html
Pathology of Laboratory Animals	http://www.AFIP.org/CLDavis/index.html
General Mouse Tumor Pathology	http://www.tumor.informatics.jax.org
Mouse Genetics	http://informatics.jax.org
NCI Mouse Models of Human Cancer Consortium (MMHCC)	http://emice.nci.nih.gov/emice/nikitin/appendix/index.html
NCI Veterinary Pathology	http://www.ncifcrf.gov/vetpath/
Strain Specific Differences; Mouse Phenome Database	http://aretha.jax.org/pub-cgi/phenome/mpdcgi?rtn=docs/home
Transgenic Histopathology	http://www-mp.ucdavis.edu/tgmouse.html
A Tumor Atlas: Information, Resources, Images and Forms	http://bioscience.igh.cnrs.fr//atlases/tumpath/index.htm
Tumor Board	http://www.tumorboard.com/

Organizing and Completing Projects

Successful projects are based on detailed discussions which define the goals, establish general agreement concerning expectations, costs, funding sources, and deadlines, and maintain effective communication among all project participants. It is easy to become briefly excited about possible projects, but is more challenging to carefully analyze important research questions and report the results. Although meetings between the pathologists and project principal investigators are important, more general sessions, including participation by technicians, graduate students, and postdoctoral fellows are crucial to success. It is enjoyable and stimulating to encourage informal slide review with pathologists as well as regular sessions in which there is in-depth correlation of pathologic changes with molecular findings and other phenotypic characteristics. The students and fellows need to meet the technicians in the mouse pathology program and know how to contact them. The investigators and pathologists need to work out the experimental plan, any warranted modifications in the project, cost estimates, account numbers against which the work is to be charged, and deadlines (real or perceived). A summary of the discussions containing all pertinent information is prepared and e-mailed by the program pathologist to all parties for editing, resulting in a final plan that is agreeable to everyone. If not already available, pathology protocols should be developed to address the project plans.

Understanding expectations on all sides is critically important. The pathologist should expect to be included on publications since s/he provides a high level of expertise (assuming the pathologist contributes to the grant application as well as to writing and editing the manuscripts). Certainly for large projects, a portion of the pathologist's and technician's salary should be included in grants along with supply costs. This actually strengthens the grant application by recognizing the need for a pathologist and documenting her/his commitment to the project. Alternative support can come from pathology cores within program project or center grants.

Training and Productivity

Conceptually, mouse pathology is part of veterinary pathology. However, veterinary as well as medical pathologists frequently do not understand mouse genetics, nomenclature, correct selection of controls, and the variety of special techniques and reagents available for these types of projects. In addition, medical pathologists generally have limited understanding of mouse biology and pathology, whereas veterinary pathologists often have inadequate knowledge of human diseases, both of which are important in disease model development. Some of this background can be gained through meetings in the United States and around the world. Collaborations bring all these skills together so that in-depth learning and productive research can take place.

Postdoctoral Training

An integral component of a mouse pathology program at academic institutions is the training of pathology residents, graduate students, and postdoctoral fellows in mouse pathology. Courses in laboratory animal pathology and participation in mouse pathology workshops will provide some of the necessary background for trainees to become proficient in mouse pathology. The importance of veterinary trainees becoming knowledgeable about human diseases and of medical trainees learning about the biology and diseases of the mouse cannot be overemphasized.

Participation of a pathology trainee in a research project may partially fulfill requirements for an MSc degree. To be successful, this arrangement requires careful planning and close, ongoing interaction between the trainee and the mentoring pathologist. Through such a program, students can investigate ongoing research projects to identify those with substantial potential for doctoral dissertation work. The student's initial pathology experience can, thus, potentially lead to graduate research in the mentor's laboratory as a PhD candidate with support from the senior investigator.

Supervisory Pathologist's Career Development

There is an inherent risk for institutions in starting new programs. However, once a mouse pathology program is in place and working efficiently, it should be productive and successful because of the tremendous need for such programs. The key is to find a pathologist with excitement, passion, and dedication, and provide institutional commitment to her/his career development. Such a person will be able to collaborate productively, and will probably develop individual research interests if s/he is not overloaded with diagnostic service work and teaching. Initial studies will lead to spin-off projects that the supervisory pathologist can develop on his/her own or in conjunction with the principal investigator. The pathologist should be included on future grant applications, program projects, and research contracts for a percentage effort plus support for a technician. In our experience,

inclusion of a pathology core in the budget of program project and center grants provides support for the salary of the pathologist and technician and for supplies. Recovery of salaries from grants will make resources available to expand the program by the addition of personnel as the volume of activity increases.

Facilities

Physical needs for performing necropsies vary based on the type and amount of anticipated work. Noninfectious disease work, using specific-pathogen-free mice, will have minor requirements. The necropsy area should contain a designated laboratory bench, preferably a defined area in a chemical fume hood (to deal with noxious fixatives) or a down-draft table with adjustable lighting. The area should have a lockable cabinet to store anesthetic drugs. A biological safety cabinet is needed for necropsy of mice exposed to certain toxins and infectious agents. Access to liquid nitrogen, a refrigerator, and a –80°C freezer to store tissues should be readily available. A ventilated hood or box under or in which to trim fixed tissues is needed, and the trim area should have a large, well-ventilated storage space. Ideally, the necropsy, tissue processing, slide preparation, and tissue storage facilities should be in close proximity to avoid long-range transportation of materials.

Ancillary Services

Pathologists use a variety of other techniques and instruments to evaluate animals, and these may be available through the veterinary/medical school pathology department and/or clinics. They include radiographic and other imaging equipment that is appropriate for use in the mouse, plus clinicopathologic, immunologic, and flow cytometric techniques. Many of these tools, which are invaluable for complete and thorough evaluation of new mouse models, are now being engineered specifically for mice (8, 11, 31). Adaptation of equipment designed for other species may be necessary. Administratively, access to these instruments and expertise can be accomplished through collaborative or fee-for-service agreements or by inclusion in a mouse phenotyping core.

Medical Records

A medical records database is useful for managing cases and providing reports in a timely manner. Networking of computers will allow the technician to enter the basic case information that is accessible to the pathologist at his/her workstation. A number of simple databases are available that can keep and retrieve records, generate typed reports, and provide summaries as spreadsheets that can be e-mailed to investigators (4, 24, 25). Ideally, the ancillary services should be set up so that results can be automatically downloaded for reporting. More expensive and complicated systems are available, and these should be evaluated for the specific needs of each pathology program.

Getting Started

Initial personnel costs include a part-time technician and staff pathologist with a defined commitment to the program. The institution must define how much weight it will give to collaborative research efforts and service versus independent research in terms of career advancement, tenure, promotion, and merit raises. Equipment and facilities investment can be minimal to moderate and include necropsy facilities (laboratory bench), digital camera with macro lens and flash or copystand with flood lights suitable for gross photography (29), networked computers for technician and pathologist, ophthalmic type surgical equipment, hoods or ventilated boxes, and a microscope with imaging capability for the pathologist as well as access to a microscope with a real-time teaching setup (analog TV camera and monitor or digital camera with real-time capabilities).

The program may arrange with expert consultants in mouse pathology to visit the institution periodically and review material. Under this arrangement, the pathologist and technician would work with the basic scientists to collect and evaluate appropriate materials over periods of three to six months. During a consultant's visit, the work would be reviewed and used to design additional experiments and generate manuscripts. This arrangement requires detailed protocols and excellent communication among the pathologist, basic scientist, and consultant. The current short supply of mouse pathology experts may limit the feasibility of this approach. A promising alternative is the use of new technologies being perfected for videoconference evaluation and discussion of digital images (5, 16).

An advisory board similar to that used for program project grants may be helpful in providing advice and oversight for the program. This could be composed of several external experts in the field of mouse pathology as well as basic scientists with an interest in the program.

Funding Options

Many veterinary colleges and medical schools provide salaries as hard money if service work is one responsibility of the position. This is the most reasonable approach to initiating a program until it becomes established and other sources of income, including fees for services, begin to cover operational costs. Initially a part-time effort should be covered for the staff pathologist and technician until the viability of the program and demand are established and income supports the operational expenses. Salary ranges for board-certified veterinary pathologists are available as a guideline (10).

The National Institutes of Health provides a number of grants that can be integrated into a pathology program for pathologists and scientists at all career levels. These include the following:

- K01/K08: Career development awards for junior pathologists interested in health-related research**,** funded by several NIH Institutes and Centers (ICs). For the Special Emphasis Career Development Award (SERCA**,** K01) for graduated veterinarians dedicated to biomedical research careers, see http://www.ncrr.nih.gov/compmed/serca.asp or http://grants1.nih.gov/grants/guide/pa-files/PA-00-019.html. For the Mentored Clinical Scientist Development Award (K08) in support of outstanding clinician research scientists dedicated to biomedical research careers, see http://grants1.nih.gov/grants/guide/pa-files/PA-00-003.html.
- K26: Mouse pathobiologist award for mid-level pathologists interested in health-related research on mouse models, funded through the National Center for Research Resources (NCRR) and the National Institute of Aging (NIA) at NIH (see http://grants.nih.gov/grants/guide/pa-files/PAR-01-064.html).
- T32: Institutional training grants awarded to veterinary schools or colleges, medical schools, or other educational or research institutions to support pre-doctoral, postdoctoral,

or short-term health professional trainees in the health sciences or quantitative sciences. These individuals may include (but are not limited to) veterinary or medical pathologists who have completed their residencies, passed their specialty boards, and are interested in advanced training in health-related research**.** Grants are funded through NCRR/NIH (see http://grants2.nih.gov/grants/guide/pa-files/PA-02-109.html).

- T35: Short-term summer training for veterinary students to entice them into biomedical research careers. Funding is through NCRR/NIH (http://www.ncrr.nih.gov/compmed/cm_rcdtf.asp).
- R01: Hypothesis-driven, investigator-initiated research project for established health-related research investigators who may also be trained pathologists. Funding is provided by almost all NIH ICs.
- R21: Exploratory/developmental and innovative research projects for assessing the feasibility of a novel area of investigation or a new experimental system that has the potential to enhance health-related research. Projects are funded by several, but not all, NIH ICs; contact program staff or see http://grants1.nih.gov/grants/guide/pa-files/PA-03-107.html or http://grants1.nih.gov/grants/funding/r21.htm for information.
- GPP: The Graduate Partnership Program (GPP; http://gpp.nih.gov) facilitates intramural and extramural research collaborations by initiating NIH-academic partnerships for health-related research training with the goal to increase interactive collaborations between research investigators at the NIH and in academia. The Comparative Molecular Pathology Research Training Program is designed to provide cross-training in animal pathology/physiology, human cancer pathology, molecular biology, and other health-related research specialties for those with doctoral degrees in veterinary medicine and for others with an interest in comparative pathology. The program is funded by the National Cancer Institute, Center for Cancer Research (NCI/CCR; http://ccr.cancer.gov/resources/training/grad_fellowship.asp.)

Program project or center grants focus on specific diseases, organ development, or other specific topics, and integrate basic science and support services to provide an interactive program resulting in higher productivity and quality work through subsidy of service cores. A mouse pathology program is an integral part of mouse biomedical research. Support for such a core is usually a critical part of these large departmental or institutional grants. They provide partial salary support for the pathologist and technician and subsidize specific services to control the costs to individual investigators.

Foundations that focus on specific diseases will support start-up projects that focus on models for the disease or, if the pathologist is an expert in the field, will provide small grants for residents and investigators to work in such program. The National Alopecia Areata Foundation (www.alopeciaareata.com) and PXE International, Inc. (http://www.pxe.org/) are examples of patient advocacy groups that sponsor research and especially animal model development, including phenotyping. The North American Hair Research Society (www.nahrs.org) is another group that focuses on an organ and its diseases rather than a specific disease. The latter is a mixture of physicians, veterinarians, and research scientists working on hair biology. The North American Hair Research Society initiated a mentorship program in 2003 that provides small grants to enable residents and junior clinicians to work for short periods with an expert in the field. This program has been particularly useful in that it provides funds for travel, room, and board. These are major issues for people in training or early in their professional careers and are usually not addressed by most other grant programs.

Advertising the Program

It is essential to inform scientists at your own institution and nearby institutions that a mouse pathology program has been established. In addition to providing research project support, the program can be involved in monitoring colony health through studies of death losses and in providing morphologic data for strain phenotyping. It can also provide interactive on-site or video-conferenced educational programs for the scientific community at the institution that range from information on the basic biology of the mouse to mouse models of specific disease. At such gatherings, basic scientists can meet the mouse pathology program members as well as other participating experts. Publicity about the program can be distributed by e-mail, direct mailing, newsletters, or other means. In addition, organization of week-long workshops on mouse pathology will give high visibility to a mouse pathology program. Such meetings in the past have been held at The Jackson Laboratory, Cornell University School of Veterinary Medicine, Baylor College of Medicine, and other sites. One such ongoing annual series is the Pathology of Mouse Models for Human Diseases Workshop (the fourth at Purdue University School of Veterinary Medicine in 2005, and the fifth at The Fred Hutchinson Cancer Research Center in 2006). This Workshop series has a faculty of more than 20 top research scientists and pathologists and is limited to 20 pathology residents or staff pathologists. It is sponsored by a U13 grant from NCRR at NIH. The Fourth International Pathology of Genetically Engineered Mice meeting is currently being organized. This large meeting is open to the entire biomedical research community and focuses on discoveries and technological advances in the field. Detailed proceedings are available (28, 30).

Reference Sources and Integration with Databases

In addition to the various training programs offered by institutions, there is a growing number of pathology textbooks that focus on anatomy, histology, and pathology of the laboratory mouse, and even more specifically on genetically engineered mice (7, 9, 12-14, 17, 18, 22, 23, 26-28, 30). These are static resources once printed. Although subsequent editions are published sporadically, they do not provide new information on a regular basis. This obstacle has been overcome by a growing number of public and private databases online (Table 1). A few databases have support from consortiums with a great deal of infrastructure in place. These are both a reference resource as well as a resource that mouse pathology programs should actively support. The best established and most heavily used include the Mouse Genome Informatics (www.informatics.jax.org), Mouse Tumor Biology (http://tumor.informatics.jax.org/FMPro?-db=TumorInstance&-format=mtdp.html&-view), Mouse Phenome (http://aretha.jax.org/pub-cgi/phenome/mpdcgi?rtn=docs/home), and Pathbase (www. pathbase.net) databases (2, 3, 15, 20).

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