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Internet and Print Resources to Facilitate Pathology Analysis When Phenotyping Genetically Engineered Rodents

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Abstract

Genetically engineered mice and rats are increasingly used as models for exploring disease progression and mechanisms. The full spectrum of anatomic, biochemical, and functional changes that develop in novel, genetically engineered mouse and rat lines must be cataloged before predictions regarding the significance of the mutation may be extrapolated to diseases in other vertebrate species, including humans. A growing list of reference materials, including books, journal articles, and websites, has been produced in the last 2 decades to assist researchers in phenotyping newly engineered rodent lines. This compilation provides an extensive register of materials related to the pathology component of rodent phenotypic analysis. In this article, the authors annotate the resources they use most often, to allow for quick determination of their relevance to research projects.

Keywords

functional genomics, Internet, mouse, phenogenomics, phenotype, rat, reference, website

In the past 25 years, genetically engineered mice (GEM) and rats (GER) have advanced from novelty status to essential tools in biomedical research programs.[167,168,220] The driving force behind this evolution is the conviction that the functional and structural consequences of altering a rodent gene will be mirrored in human patients who carry a similar genetic anomaly in the homolog gene.[204] Supporting the accuracy of this premise is the fact that the effects in human patients of the 100 top-selling pharmaceutical agents are predicted by the GEM phenotypes that arise when genes encoding each drug's target are deliberately disrupted.[245] Another development in genetic engineering during the last decade has been the generation of humanized rodent models (ie, substitution of a human molecule or cell type in place of its native rodent counterpart), thus providing a system to test the functional and structural significance of human molecules in vivo.[168,243] The push to annotate the whole mammalian genome by comprehensive gene targeting in GEM[30,130,193] will greatly expand the magnitude of engineered rodent research and its application to human disease modeling.

Proficient phenotyping of GEM and GER requires regular access to a range of biomedical expertise. The complexity of phenotypic analysis typically necessitates a team approach for greatest success. In many cases, such know-how is provided within an institution through the assembly of "comprehensive centers" or "shared phenotyping resources" comprising several collaborating research and/or service laboratories. In other instances, the entire institute is fully dedicated to rodent phenotyping. Either strategy is suitable as long as a critical mass of multidisciplinary expertise is available to support the efforts of the research team conducting its experiments.

The key bottleneck affecting all rodent phenogenomic programs, including the international effort to annotate the mammalian genome,[130] is the inability to perform high-throughput phenotypic analysis at a pace that matches the generation of new GEM and GER lines. A chief factor in this phenotyping backlog is the well-documented dearth of experienced comparative pathologists.[13,35] This shortage is the outcome of 2 primary trends in scientific research. First, laboratory animal pathologists are usually allocated to other research roles, such as disease diagnosis in laboratory animal facilities or evaluation of rodent tissues from toxicity bioassays. More important, training programs designed to produce comparative pathologists with substantial experience in rodent-based research are uncommon, so most individuals who enter the field do so at present by undertaking an informal course of mentored or self-directed independent study.[24] The major flaw of this individualized approach to comparative pathology training is that,

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Table 1. Essential Printed Resources for Comparative Pathologists Engaged in Phenotypic Analysis of Engineered Rodents

Title	First Author/Editor	Type	Citation
Analytic methods			
"Evaluating Mutant Mice: Anatomic Pathology" This brief review provides a succinct introduction to major considerations required to phenotype rodents. Emphasis is placed on basic knowledge and skills required for structural analysis of adult animals.	Brayton	Article	27
Mouse Phenotypes: A Handbook of Mutation Analysis This book is a good introduction regarding procedures used to generate and assess prenatal and postnatal phenotypes of mice.	Papaioannou	Book	171
Systematic Approach to Evaluation of Mouse Mutations This volume describes the basic characteristics of building and maintaining mouse colonies, as well as techniques used to evaluate mutation-induced phenotypes.	Sundberg	Book	216
Anatomy			
Anatomy of the Rat This guide provides a detailed, well-illustrated description of rat organs and systems. Most of the information can be applied to mice.	Greene	Book	90
Colour Atlas of Anatomy of Small Laboratory Animals This 2-volume set includes detailed color plates showing major anatomic features for many rodent species, including mice and rats.	Popesko	Book[a]	187
Biology of rodents			
The Laboratory Mouse This book reviews major aspects of mouse-based research, including history, husbandry, strain nomenclature, breeding, anatomy, physiology, pathology, pathogens, genomics, procedures, and safety study design.	Hedrich	Book	96
The Laboratory Rat This reference reviews most aspects of rat-based biomedical research, including history, husbandry, strain nomenclature, breeding, anatomy, physiology, procedures, and design of various safety studies.	Krinke	Book	135
The Laboratory Rat One of the "Blue Book" volumes commissioned by the American College of Laboratory Animal Medicine, this volume provides a detailed overview of rat biology, including multiple chapters on various disease conditions.	Suckow	Book	213
The Mouse in Biomedical Research, 2nd ed.	Fox	Books	
Vol 1: History, Wild Mice, and Genetics			70
Vol 2: Diseases			71
Vol 3: Normative Biology, Husbandry, and Models			72
Vol 4: Immunology These 4 American College of Laboratory Animal Medicine "Blue Books" provide encyclopedic coverage of mouse biology and pathology, as well as the place of mouse models of disease in biomedical research.			73
Clinical pathology			
The Clinical Chemistry of Laboratory Animals This resource offers an extensive review of principal clinical chemistry analyses and their interpretation from multiple laboratory animal species, including mice and rats.	Loeb	Book	140

(continued)

Table 1. (continued)

Title	First Author/Editor	Type	Citation
Schalm's Veterinary Hematology The volume provides a thorough overview of blood collection and analytic methods for many species, including detailed chapters on mouse and rat blood.	Weiss	Book	235
Development			
The Atlas of Mouse Development, 2nd ed. This book is the premier photomicrographic atlas for normal mouse anatomy and multiple gestational stages. Images mainly represent 2-dimensional step sections of embryos and fetuses, but some 3-dimensional plates are included to demonstrate progressive development over time of select organs.	Kaufman	Book	127
Manipulating the Mouse Embryo, 3rd ed. This volume is an encyclopedic collection of methods used to produce genetically engineered mice, but it incorporates many standard pathology methods for morphologic analysis of mouse embryos and fetuses.	Nagy	Book	159
Pathology of the Developing Mouse: A Systematic Approach This book provides a methodical review of the anatomic pathology and clinical pathology techniques used for the structural analysis of mouse embryos, fetuses, and placentae. The unique feature is that patterns of common developmental defects are described and illustrated.	Bolon	Book	18
Pathology			
Mouse			
International Classification of Rodent Tumours: The Mouse This book illustrates and describes the typical features of proliferative lesions in mice (both neoplastic and nonneoplastic). Entries include major differential diagnoses.	Mohr	Book	150
Pathobiology of the Aging Mouse This 2-volume set provides pathology data for many conditions of mature and senescent mice, arranged by systems. Both nonproliferative and neoplastic lesions are addressed.	Mohr	Book[a]	152
Pathology of Genetically Engineered Mice This book gives an overview of the primary analytic methods used to assess genetically engineered mice for prenatal and postnatal phenotypes, with chapters describing common phenotypes for many organ systems.	Ward	Book[a]	233
Pathology of Laboratory Rodents and Rabbits, 3rd ed. This manual describes and illustrates the main lesions produced by major rodent pathogens while listing the main differential diagnoses for these diseases.	Percy	Book	185
Pathology of the Mouse: Reference and Atlas This tome supplies a thorough review of the anatomy and pathology for major organ systems in the mouse. Coverage is provided for degenerative, neoplastic, and toxicant-induced lesions as well as common incidental findings.	Maronpot	Book	145
Rat			
Handbook of Toxicologic Pathology This 2-volume set contains system-specific chapters packed with normal and toxicant-altered anatomic and functional features of laboratory animals, especially rodents.	Haschek	Book	94
Monographs on Pathology of Laboratory Animals This multivolume collection describes system-specific pathology in rats and mice. Normal anatomy and nonproliferative and neoplastic lesions are discussed.	Jones	Book[a]	

(continued)

Table 1. (continued)

Title	First Author/Editor	Type	Citation
Cardiovascular and Musculoskeletal Systems			118
Digestive System			124
Endocrine System			115
Eye and Ear			119
Genital System			120
Hematopoietic System			123
Integument and Mammary Glands			121
Nervous System			122
Respiratory System			116
Urinary System			117
Pathobiology of the Aging Rat This 2-volume set provides pathology data for many conditions of mature and senescent rats, arranged by systems. Both nonproliferative and neoplastic lesions are addressed.	Mohr	Book[a]	151
Pathology of the Fischer Rat: Reference and Atlas This tome supplies a thorough review of the anatomy and pathology for major organ systems in the rat. Coverage is provided for degenerative, neoplastic, and toxicant-induced lesions as well as common incidental findings.	Boorman	Book[a]	20
Organ-specific resources			
Nervous system			
The Mouse Brain in Stereotaxic Coordinates, 3rd ed. This atlas shows well-annotated step sections of mouse brain features in all 3 dimensions.	Franklin	Book	74
The Rat Brain in Stereotaxic Coordinates, 6th ed. This atlas shows well-annotated step sections of rat brain features in all 3 dimensions. Individuals who perform rat neuropathology assessments on a regular basis may desire access to earlier editions, which present the intervening step sections.	Paxinos	Book	180
Reproductive tract			
"The Female Rat Reproductive Cycle: A Practical Histological Guide to Staging" This article provides a good review of the morphologic variations of different reproductive tract locations over the entire estrous cycle.	Westwood	Article	239
Strain-specific physiology			
"Genetic Variation Among 129 Substrains and Its Importance for Targeted Mutagenesis in Mice" This article is a powerful reminder that insufficient care in mouse husbandry can have substantial impact on genetic backgrounds and the availability of suitable control animals. The piece is important because many targeted mutations ("knockout" lines) are generated using 129 substrains.	Simpson	Article	208
"Pathology of Aging B6;129 Mice" This article presents primary lesions that frequently affect mice with this genetic background, as targeted mutations commonly introduce 129-derived embryonic stem cells into C57BL/6 blastocysts.	Haines	Article	92
"Spontaneous Lesions in Aging FVB/N Mice" This article presents primary lesions that frequently affect mice with this genetic background, as many transgenic lines are made by microinjection of engineered DNA into FVB/N oocytes.	Mahler	Article	143

[a]Out-of-print reference.

Table 2. Essential Internet Resources for Comparative Pathologists Engaged in Phenotypic Analysis of Engineered Rodents

Title	Citation
Mouse	
"Comparative Placentation," http://placentation.ucsd.edu/	15
A comprehensive site with text and images that compares placental structure among many species, including rodents. The simplest means for accessing mouse and rat data is to use the "Index" button and then scroll to the bottom to find the "Rodentia" section.	
"Inbred Strains of Mice," http://www.informatics.jax.org/external/festing/mouse/STRAINS.shtml	67
This venue provides an alphabetical listing of major mouse strains with links to extensive documentation regarding biological characteristics. This site offers a thorough overview of strain-specific anatomy and physiology.	
"Mouse Brain Library," http://mbl.org/	241
This site catalogs high-resolution images and databases of brains from several mouse strains. Atlases are available with sections in 2 orientations (coronal and horizontal) for various ages.	
"Mouse Genome Informatics," http://www.informatics.jax.org	106
This portal contains genomic and phenotypic data for many mouse strains. Links provide ready access to searchable databases that match mutations (engineered and spontaneous) to phenotypes, describe gene maps and markers, and allow comparison of genomic information among species.	
"Mouse Nomenclature," http://www.informatics.jax.org/mgihome/nomen/index.shtml	107
This portal provides entry to rules and guidelines for naming genes, alleles, mutations, chromosomal aberrations, and mouse and rat strains.	
"Pathology of Genetically Engineered Mice," http://www.niaid.nih.gov/labsandresources/labs/aboutlabs/cmb/infectiousdiseasepathogenesissection/vetpathology/geneticallyengineeredmice/Pages/geneticallyEngineeredMice.aspx	166
This clearinghouse provides links to numerous sites of relevance to mouse pathologists, including a link to a companion site dealing with rodent immunohistochemistry.	
Pathology nomenclature	
These collections provide a comprehensive list of lesion names and descriptions for common abnormalities in major organs of mice and rats. The older Standardized System of Nomenclature and Diagnostic Criteria guides are currently being replaced by the INHAND documents.	
"Standardized System of Nomenclature and Diagnostic Criteria (SSNDC) Guides," http://www.toxpath.org/ssndc.asp	211
"International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice," http://www.toxpath.org/inhand.asp	104
Phenome databases	
Taken together, these portals offer a comprehensive compilation of mouse physiologic data that can be searched by strain, organ system, or biological parameter. Links to protocols for acquiring such data are also given.	
"Europhenome," http://www.europhenome.org/databrowser/viewer.jsp	64
"Mouse Phenome Database," http://phenome.jax.org/	108
"Pathbase," http://www.pathbase.net/	6
"Phenotyping of Genetically Engineered Mice," http://dels-old.nas.edu/ilar_n/ilarjournal/47_2/html/	157
The site provides access to the free online archive of an *ILAR Journal* issue discussing methods and policy considerations to contemplate when generating and analyzing mouse colonies with genetically engineered phenotypes.	
"Revised Guides for Organ Sampling and Trimming in Rats and Mice," http://reni.item.fraunhofer.de/reni/trimming/index.php	158
This site gives access to guides for organ sampling and trimming in mice and rats.	
"Visible Mouse Project," http://tvmouse.compmed.ucdavis.edu/	81
The site is a clearinghouse for multimedia presentations describing the anatomy, physiology, histology, and pathology of the laboratory mouse, emphasizing the features of genetically engineered mice.	
Rat	
"Rat Genome Database," http://rgd.mcw.edu/	109
This portal affords entry to information regarding many aspects of rat biology, including links to genomic, proteomic (pathway), functional, phenotypic, and strain data.	

by definition, it fails to provide a standard knowledge base for individuals engaged in GEM and GER phenotyping projects.

Efforts are currently underway by several organizations (eg, the Academy of Genomic Pathology, http://ctrgenpath.net/?page_id=2612; the ACVP/STP Coalition for Veterinary Pathology Fellows, http://www.vetpathcoalition.org/) to produce more consistent training experiences in comparative pathology emphasizing rodent phenotypic analysis. However, the curricula and final product (eg, formal degree, continuing education certificate) have yet to be defined. Therefore, individuals interested in acquiring knowledge and practical skills in comparative pathology must continue to pursue their education in a self-directed fashion for the present.

A partial remedy for the scarcity of organized training programs in rodent pathology is to devise a self-study program based on readily available resources. The current review is designed to be a multidisciplinary compilation of Internet and print resources to support a well-rounded autotutorial

Table 3. Age-, Disease-, and Organ-Specific References to Aid in the Phenotypic Analysis of Engineered Rodents

Subject	Citation
Anatomy	42, 45, 95, 134, 187, 207, 231
Anatomic pathology	
Macroscopic assessment	27, 66, 68
Microscopic analysis	4, 44, 87, 131, 153, 196, 209
Clinical pathology	
Baseline physiological parameters	31, 32, 137, 146, 188, 222
Sample collection	41, 100, 146
Development	
Analytical methods	10, 29, 46, 55, 159, 160
Anatomy	2, 3, 47, 49, 110, 111, 148, 172, 173, 175, 186, 198–200, 214, 221, 230, 234
Biology	52, 63, 78, 79, 85, 88, 95, 128, 129, 190, 191, 195, 197, 201, 242, 248–250
Pathology	83, 226
Disease	
Cancer	5, 17, 33, 77, 91, 103, 105, 132, 150, 154, 162, 163, 169, 203, 212, 236
Infections	8, 9, 71, 147, 213
Genetics	70, 206
Models	164
Nomenclature	139, 142
Pathology	20, 87, 89, 145, 185, 233
Phenotyping	
General	11, 12, 22, 28, 53, 102, 149, 165, 180, 217
Influence of genetic background	11, 139
Behavior	48, 155, 192
Blood	62, 65, 75, 112, 132, 146, 154, 224, 235
Cardiovascular	
Analytical methods	93, 141, 244
Anatomy	198, 232, 234, 238
Biology	114, 138
Digestive	17, 103
Endocrine	126
Eye	40, 210
Immune system	14, 38, 39, 56–61, 73, 76, 144, 154, 183, 184, 202, 218, 224, 225, 229, 240
Mammary gland	33, 34, 98
Nervous system	
Neuroanatomy	1–3, 7, 25, 50, 54, 74, 99, 133, 136, 173–181, 199, 200, 205, 219, 228, 237, 241, 246, 247
Neurochemistry	86, 111, 182, 223
Neurology	26, 50
Neurophysiology	43
Neuropathology	19, 69, 82, 84, 91, 125, 212, 236
Tissue preparation	36, 69, 97, 170
Placenta	51, 113, 161, 194
Pulmonary	141, 169
Reproductive	21, 203
Skeleton	23
Skin	156, 215
Urinary	141
Physiology	16, 37, 101, 114, 141, 189, 227
Statistics	80

curriculum in GEM and GER pathobiology and phenotyping. The cited resources are arranged in 3 tables for convenience. The first 2 tables reflect the printed materials (eg, books and journal articles; Table 1) and websites (Table 2) that we most commonly use in our daily course of comparative pathology research. Each frequently utilized reference is followed by a brief description of its content and main features. We recommend that the annotated print items in Table 1 be available in each pathologist's departmental library, and ideally in his or her office. The third table lists topics related to rodent phenotyping that occasionally require input from a comparative pathologist but fall outside the core pathology expertise expected of such individuals. These latter materials are accompanied by a list of one or more nonannotated citations that offer further discussion on such auxiliary subjects. For some topics in Table 3 (eg, neuroanatomy atlases for adult mice), multiple editions of a given book have been produced by the same authors. All editions have been included in the citation list in cases where we have found that the various versions may illuminate distinct features of the given rodent system.

A solid understanding of the foundational knowledge required for proficient GEM and GER phenotyping may be readily attained by a sincere study of the resources in the annotated lists. Sufficient time will have to be spent perusing these baseline resources so that the budding comparative pathologist becomes familiar with not only individual facts but also the location of essential topics of relevance to phenotyping GEM and GER models. Pathologists who regularly participate in rodent phenotyping will also need to engage in a lifelong quest for continuing education due to the rapid evolution of information, skills, tools, and techniques within this field.

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