1

Introduction to Mouse Pathology

Jerrold M. Ward, Peter Vogel, and John P. Sundberg

The Use of Mice in Medical Research

There has been a large increase in the use of mice in both basic and translational medical research over the past 20 years, as evidenced by the increase in retrievable references using "mice" as a keyword in PubMed from 29 029 publications in 1998 to 79 858 in 2018, and over 1.6 million in the entire PubMed database. Mice have been used for both basic and translational (formerly applied) research. Much of this important work is sponsored by the National Institutes of Health (NIH) in the United States. Mice have been used to study basic biological processes, embryo development, genetic disorders, infectious diseases, degenerative diseases, toxicology, carcinogenesis, and aging, often by organ system or tissue. Many Nobel Prize awardees in Physiology or Medicine have used mice in research leading to their honors. Much of this research has been greatly valued for the training of future scientists, discovery of new diseases, understanding the mechanism of disease in mice and other animals, including humans, and in the treatment and prevention of disease in mice and humans [1–8]. Histopathology may be included in the mouse research, but often it is not, or not by someone trained and competent in pathology [9]. The value of pathology has been proven for diagnosis and understanding normal biology and abnormal biology (pathology) of cells, tissues, and organs in all species. Comparative pathology spans all species of animals. But some investigators do not understand the value of pathology, as a discipline, in experimental studies with mice. This book intends to promote the value of mouse pathology in medical research aimed at the discovery of the causes, prevention, and therapy of diseases in both humans and other animals.

Understanding Diseases Found in Mutant Animals

Naturally occurring and induced changes in genes often result in a specific phenotype at the clinical and histopathological levels. The cause of this phenomenon is that genes have specific functions that play a role in the normal homeostasis of cells, tissues, and organs. A genetically engineered mouse (GEM) line represents a tool to investigate the effects resulting from the partial or complete loss of gene function or the gain of normal or abnormal functions. The genes are often found to function in specific cellular organelles and biochemical/molecular pathways important for normal biological functions (Figure 1.1) [10]. Cells involved in common gene functions can be single cell types in single tissues, multiple cells types in a single tissue, or multiple cells types in multiple tissues. Gene expression can also be induced in specific cells and tissues by normal and abnormal body functions, or by exposure to external factors such as drugs, infectious agents, environmental modifications, and ingested foods.

Mutant mice often exhibit histopathological changes (lesions) in tissues and organs that are associated with gene function, but the ultimate clinical phenotype can be influenced by various factors. Much of the histopathology found in mutant mice is in the usual spectrum of degenerative, inflammatory, proliferative, and neoplastic changes also found in nonmutant mice. Nouvelle lesions do occur commonly in some lines of mutant mice. These include unique developmental changes, cellular morphological changes, patterns of lesions, types of proliferative lesions, and often strain specific cancer types. These may be due to specific naturally occurring mutations (polymorphisms) in genes

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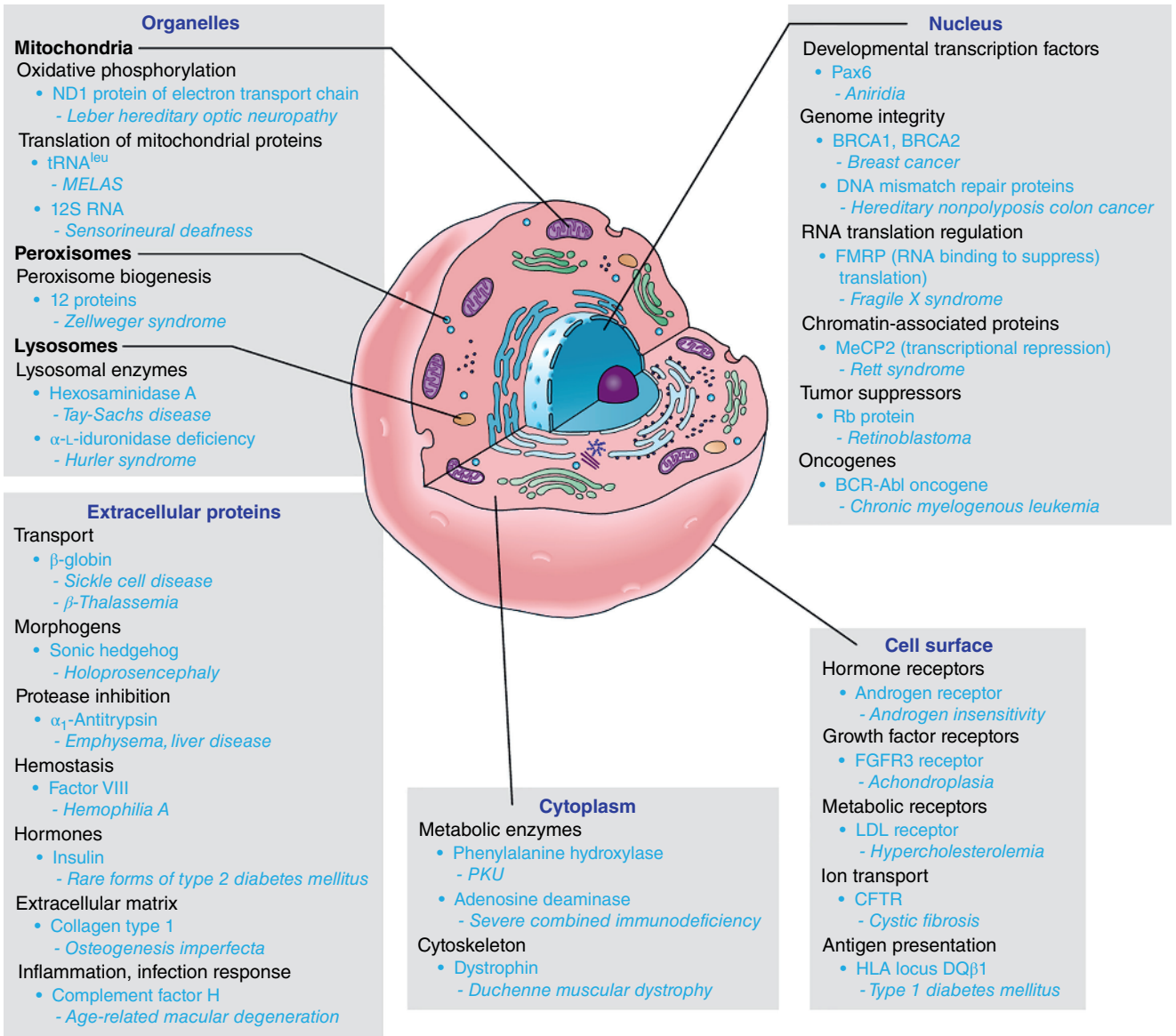

Figure 1.1 Classes of proteins associated with human genetic diseases. Source: Nussbaum (2007). Reprinted with permission of Elsevier

that can serve as genetic based models for disease. Spontaneous mutants made up the bulk of mouse models until the advent first of radiation and then chemical mutagenesis programs. The spectrum of spontaneous lesions is noted in each organ-specific chapter. Often the lesions are identical or very similar to those associated human genetic disorders, but they also may vary from human lesions. The genetic background of the mice often plays a role in spontaneous as well as induced disease phenotypes.

Mouse Pathology – Nomenclature

The pathology of mice in research was first led by Thelma Dunn and Harold Stewart at the National Cancer Institute (NCI) at the NIH [11–13]. Both were MD pathologists who applied general rules of human pathology diagnosis to mice when possible. Although mouse pathology nomenclature does not follow any official designation, two organizations (INHAND; https://www.toxpath.org/inhand.asp and National Cancer Institute Mouse Models of Cancer Consortium Tumor Pathology Nomenclature) have provided international nomenclatures for specific tissues [2, 14]. Other published guides are included in each appropriate organ chapter [15, 16]. Many books and refereed publications on mouse pathology provide valuable information for pathologists and scientists [12, 17–35] as well as web sites (https://ntp.niehs.nih.gov/nnl; http://www.informatics.jax.org/frithbook; http://eulep.pdn.cam.ac.uk/~skinbase/index.php). Importantly, many of these references involve both DVMs and MDs, pathologists, and basic scientists, who integrate mouse and human disease nomenclature together to be state-of-the-art. The pathology nomenclature used in this

book generally reflects the NCI tumor pathology and INHAND general pathology nomenclatures. There are, however, no international or national standards for nomenclature that must be followed. These published nomenclatures are merely guidelines for use by scientists, pathologists, and journals. Each chapter author considered these guidelines and noted appropriate references for each organ and tissue.

Mouse Genetic Nomenclature

In contrast to pathology nomenclature, mouse genetic nomenclature is standardized. Chapter 3 focuses on the details of the nomenclature system and discusses how it was developed. While the authors and editors have, for the most part, updated the nomenclature, not all authors were willing to do so. Regardless, one can and should use the Mouse Genome Informatics website to verify all genes and alleles, as discussed in the Chapter 3, to make sure they are working with the correct nomenclature and allelic mutations.

Tumor Pathology

It is known in human and mouse pathology that cancer pathogenesis follows a scheme of molecular pathogenesis and an associated histopathogenesis [14, 16, 36]. There have been numerous publications on the role of specific genes in tumor pathogenesis in humans and animals. It is not the intention of this book to review the role of all genes for which published information on mouse cancer models is available, but rather to provide samples of some of the more common and important genes that play important roles. GEM may involve a single gene and attempt to mimic the human genetic disorder, or GEM may represent non-familial genetic changes in the pathways to disease including cancer. Tumor frequency data in wild-type control mice, especially in aging studies, have often been reported in various strains and stocks [1, 7, 23, 35, 37, 38]. While these reports provide general background information on the frequency of cancer types in a wildtype inbred strain, the actual frequency will vary based on substrain, husbandry, and other factors, necessitating the use of adequate numbers of control mice for studies on frequency of cancers in GEMs.

Immunohistochemistry (IHC), Scoring, Image Analysis, and Other Supportive Research Pathology Techniques

A variety of special pathology techniques are important adjuncts to mouse research. These include immunohistochemistry (IHC), *in situ* hybridization (ISH), ultrastructure, imaging, image analysis, artificial intelligence, machine learning, and a variety of molecular techniques. Most chapters will include examples of these for the various tissues. Some publications offer reviews of the use of IHC in mice [39–42] and Internet sites offer IHC protocols (http://tumor.informatics.jax.org/mtbwi/index.do, https://www.niehs.nih.gov/research/resources/protocols/protocols-immuno/index.cfm) and whole slide images (http://tumor.informatics.jax.org/mtbwi/lymphomaPathology.jsp).

Histopathology scoring (grading) of lesion type and severity can often be used for mouse models of disease, genetics, and preclinical development for drugs [6, 34, 43–45]. Examples are given in some chapters. The newer fields of image analysis, artificial intelligence, and machine learning are growing quickly [46, 47] and allow scientific analysis of quantitative pathology data.

Publication of Erroneous Pathology Data: Inadvertent Fraud?

Publications involving mice and other animals sometimes include histopathology figures that do not show what is described in the figure legend and/or text [2, 48, 49]. This problem has occurred most often in publications that do not appear to include a pathologist as a co-author, and in journals that are not pathology-based. The absence of pathology support at a research institution may be due to cost, lack of pathology staff, and/or the desire of a scientist and his staff to attempt pathology on their own ("Do It yourself pathology") [50]. These publications containing clearly erroneous findings may be due to lack of pathologists as reviewers of the submitted manuscript and/or a lack of the journal reviewers and editors who understand the value of accurate histopathology description and interpretation. Emails to authors and editors involved with the publication usually evoke no responses, and rarely concern even if there is a response. The only solution is for scientists and journals to understand the importance of pathology as a critically important medical specialty as part of doing research using mice.

Overall Organization of the Book

The *Pathology of Genetically-engineered Mice (GEM) and Other Mutant Mice* is organized into introductory chapters on concepts on the use of mice in biomedical research and the critical need to include mouse pathology expertise. The mouse pathology chapters, by organ system or other topics, are generally organized into sections on anatomy and histology, special necropsy organ protocols, aging/spontaneous strain specific lesions, GEM, and references. The reference listings are not an extensive review of the topic since there are thousands of such references, but they do include recent reviews and relevant classic and current publications. Tables and figures call attention to important information on the mice and their applications.

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