Pathology: the clinical description of human disease

William K. Funkhouser

Department of Pathology and Lab Medicine, UNC School of Medicine, Chapel Hill, NC, United States

Summary

Pathology is that field of science and medicine concerned with the study of diseases, specifically their initial causes (etiologies), their step-wise progressions (pathogenesis), and their effects on normal structure and function. This chapter will consider the history of relevant discoveries and technologies that have led to our current understanding of diseases, as well as the Pathologist's current role in the diagnosis, prognosis, and prediction of response of human diseases.

> "Future discoveries will not *likely be made by morphologists ignorant of molecular biologic findings, or by biologists unaware or scornful of morphologic data, but by those willing and capable of integrating them through a team approach . . ."*
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> Rosai J. Rosai and Ackerman's surgical pathology. 9th ed. St. Louis, MO: Mosby; 2004.

Introduction

This chapter will discuss the fundamental concepts, terminology, and practice of pathology as the discipline dedicated to the understanding of causes, mechanisms, and effects of diseases. A section on key terms, definitions, and concepts is followed by sections on historical human approaches to diseases, an overview of current diagnostic practice, and a vision for new interface with applied molecular biology.

Terms, definitions, and concepts

Pathology (from the Greek word *pathología*, meaning the study of suffering) refers to the specialty of medical science concerned with the cause, development, structural/functional changes, and natural history associated with diseases. *Disease* refers to a definable deviation from a normal phenotype (observable characteristics due to genome and environment), evident via patient complaints (symptoms), and/or the measurements of a careful observer (signs). The cause of the disease is referred to as its *etiology* (from the Greek word meaning the study of cause). One disease entity can have more than one etiology, and one etiology can lead to more than one disease. Each disease entity develops through a series of mechanistic chemical and cellular steps. This stepwise process of disease development is referred to as its *pathogenesis* (from the Greek word meaning generation of suffering). Pathogenesis can refer to the changes in the structure or function of an organism at the gross/clinical level, and it can refer to the stepwise molecular abnormalities leading to changes in cellular and tissue function.

The presentation of a disease to a clinician is in the form of a human patient with variably specific complaints (*symptoms*), to which the examining physicians can add diagnostic sensitivity and specificity by making observations (screening for *signs* of diseases). These phenotypic (measurable characteristic) abnormalities reflect the interaction of the genotype (cytogenetic and nucleic acid sequence/expression) of the patient and his/her environment. Patient *workup* uses present illness history with reference to past medical history, review of other organ systems for other abnormalities, review of family history, physical examination, radiographic studies, clinical laboratory studies (for example, peripheral blood or CSF specimens), and anatomic pathology laboratory studies (for example, tissue biopsy or pleural fluid cytology specimens). As you will see from other chapters in this book, the ability to rapidly and inexpensively

screen for chromosomal translocations, copy number variation, genetic variation, and abundance of mRNA and miRNA is adding substantial molecular correlative information to the workup of diseases.

The *differential diagnosis* represents the set of possible diagnoses that could account for symptoms and signs associated with the condition of the patient. The conclusion of the workup generally results in a specific diagnosis which meets a set of diagnostic criteria, and which explains the patient's symptoms and phenotypic abnormalities. Obviously, arrival at the correct diagnosis is a function of the examining physician and pathologist (fund of knowledge, experience, alertness), the prevalence of the disease in question in the particular patient (age, race, sex, site), and the sensitivity/specificity of the screening tests used (physical exam, vital signs, blood solutes, tissue stains, genetic assays). The *pathologic diagnosis* represents the best estimate currently possible of the disease entity affecting the patient, and is the basis for downstream follow-up and treatment decisions. The diagnosis implies a natural history (course of disease, including chronicity, functional impairment, and survival) that most patients with this disease are expected to follow. Be aware that not all patients with a given disease will naturally follow the same disease course, so differences in patient outcome do not necessarily correspond to incorrect diagnosis. Variables that independently correlate with clinical outcome differences are called *independent prognostic variables* , and are routinely assessed in an effort to predict the natural history of the disease in the patient. It is also important to note that medical therapies for specific diseases do not always work. Variables that independently correlate with (predict) responses to therapy are called *independent predictive variables*.

Diagnosis of a disease and development of an effective therapy for that disease do not require knowledge of the underlying etiology or pathogenesis. For example, granulomatous polyangiitis (née Wegener's granulomatosis) was understood by morphology and outcomes to be a lethal disease without treatment, yet responsive to cyclophosphamide and corticosteroids, before it was found to be an autoimmune disease targeting neutrophil cytoplasmic protein PR3 (Fig. 11.1). However, understanding the molecular and cellular pathogenesis of a disease allows development of screening methods to determine risk for clinically unaffected individuals, as well as mechanistic approaches to specific therapy.

The Pathologist is that physician or clinical scientist who specializes in the art and science of medical risk estimation and disease diagnosis, using observations at the clinical, gross, body fluid, light microscopic, immunophenotypic, ultrastructural, cytogenetic, and molecular levels. Clearly, the pathologist has a duty to master any new concepts, factual knowledge, and technology that can aid in the estimation of risk for unaffected individuals, the statement of accurate and timely diagnosis, accurate prognosis, and accurate prediction of response to therapy for affected individuals.

A brief history of approaches to disease

The ability of *H. sapiens* to adapt and thrive has been due in part to the ability of humans to remember the past, respect tradition, recognize the value of new observations, develop tools/symbols, manipulate the environment, anticipate the future, and role-specialize in a social structure. The history of human understanding of diseases has progressed at variable rates, depending on the good and bad aspects of these human characteristics.

Concepts and practices before the scientific revolution

Our understanding of ancient attitudes toward diseases is limited by the historical written record. Thus, the start point for written medical history corresponds to around 1700 BCE for Mesopotamian rules in the code of Hammurabi, and around 1550 BCE for the analogous Egyptian rules in the Ebers papyrus. By definition, these philosophers, theologians,
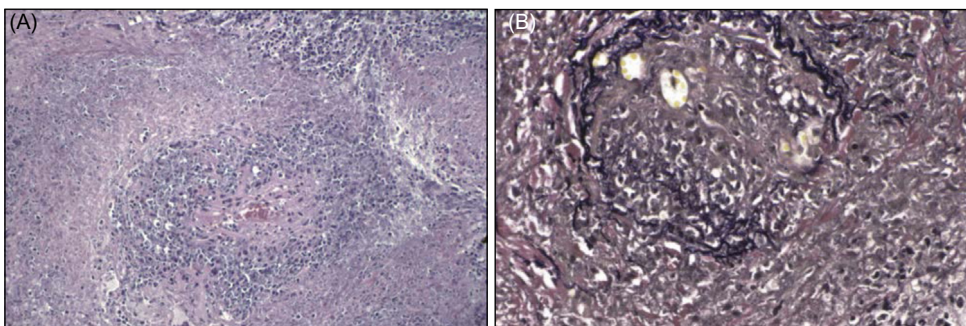

FIGURE 11.1 Granulomatous polyangiitis (née Wegener's granulomatosis) of the lung. (A) Hematoxylin and eosin staining of granulomatous polyangiitis of lung. Necrosis, granulomatous inflammation, and vasculitis are identified. (B) Elastin stain of granulomatous polyangiitis of lung. Elastica disruption of the arterial wall supports a diagnosis of vasculitis.

and physicians had access and assets to allow a written record, and materials and storage sufficient for the written records to survive. The Mesopotamian records indicate a deity-driven, demon-driven theory and empirical practice by recognized professional physicians. In this context, the prevailing thought was that "*Disease was caused by sprit invasion, sorcery, malice, or the breaking of taboos; sickness was both judgment and punishment . . .*"

The Greek medical community evolved a theory of disease related to natural causes and effects, with less emphasis on deity-driven theory. The Hippocratic Corpus includes "On the Sacred Disease" (circa 400 BCE), which rejected a divine origin for diseases, and postulated a natural rather than supernatural basis for disease etiology ("*. . . nowise more divine nor more sacred than other diseases, but has a natural cause . . . like other affections. . .*"). Aristotle (384−322 BCE) wrote broadly on topics including logic, biology, physics, metaphysics, and psychology. To Aristotle, observations led to a description of causes, or first principles, which in turn could be used logically in syllogisms to predict future observations. We would agree with these basic notions of induction and deduction. However, there was a different background philosophical construct regarding the nature of matter and causality (four elements, four humors, and four causes, including a final or teleological purpose). We would recognize Aristotle's "efficient" cause of a disease as its etiology. Alexander the Great's conquest of Egypt in the 4th century BC led to Greek (Ptolemaic) leadership of Egypt from 305 BCE to 30 BCE, with development of the Alexandrian library and University. Faculty such as Euclid developed geometric models of vision (*Optica*), and Herophilus described human anatomy by direct dissection and observation (Greek medicine apparently allowed dissection in Alexandria, including vivisection of the condemned). During the Roman imperial era, Galen (129−207 CE) used dissection and observation of other animals such as the macaque (human dissection was illegal) to extrapolate to human anatomy and physiology. Like Aristotle's approach, Galen's approach to patients, diseases, and treatments was guided by philosophical constructs of four humors (blood, phlegm, yellow bile, and black bile) and the resulting temperaments (buoyant, sluggish, quick-tempered, and melancholic) due to humoral imbalances. It is thought that many of Galen's texts were destroyed with the Alexandrian library before the 7th century AD, but a subset was preserved and translated by Middle Eastern scholars. These ancient classic texts were then retranslated into Latin and Greek when printing houses developed in the 15th century (for instance, Hippocrates' *De Natura Hominis*, circa 1480 CE, and Galen's *Therapeutica*, circa 1500 CE).

The historical picture of the Greco-Roman understanding of disease is one of empirical approaches to diseases based on inaccurate understanding of anatomy, physiology, and organ/cellular pathology. Greek medicine became less superstitious and more natural cause-and-effect oriented, yet philosophy still trumped direct observation, such that evidence was constrained to fit the classic philosophical constructs. Some of the concepts sound familiar; for example, normal represents equilibrium and disease represents disequilibrium. However, we would differ on what variables are in disequilibrium (the historical humors, numbers, and opposites versus contemporary chemical and kinetic equilibria).

Following the collapse of the Western Roman Empire in 476 CE, the classic texts of Aristotle, Hippocrates, and Galen were protected, translated, and built upon in the Byzantine and Arab societies of the near East, and in Spain during the Muslim/Moorish period through the 11th century. During these middle ages for Western Europe outside Spain, there was apparently a retreat to pre-Hellenistic beliefs in supernatural forces that intervened in human affairs, with protecting saints and relics for disease prevention and therapy. Centers of medical learning following the Spanish Muslim model developed in Montpellier, France, and in Salerno, Italy, beginning in the 11th century.

The scientific revolution

Aristotle's concept of induction from particulars to general first principles, then use of syllogistic logic to predict particulars, evolved into the scientific method during the Renaissance. Ibn Alhazen's (al-Haitham's) work on the physics of optics in the 11th century challenged Euclid's concepts of vision from the Alexandrian era. (Euclid thought that the eye generated the image, rather than light reflected from the object being received by the eye). In the 13th century, Roger Bacon reinforced this use of observation, hypothesis, and experimentation. The printing press (Gutenberg, 1440 CE) allowed document standardization and reproduction, such that multiple parallel university and city libraries could afford to have similar collections of critical texts, facilitating scholarly publications in journals. Access to publications in libraries and universities led to a system of review, demonstration, discussion, and consensus regarding new scientific findings.

The concept of the body as an elegant machine was captured by not only 15th and 16th century Renaissance artists like da Vinci and Michelangelo, but also by anatomists and pathologists interested in the structure/function of health and disease. The ancient models of Aristotle and Galen had become sacrosanct, and newer, evidence-based models were considered heretical to some degree. So it was somewhat revolutionary when Vesalius dissected corpses, compared them with Galenic descriptions, and published *De humani corporis fabrica libri septem* (1543 CE; "Seven Books

on the Structure of the Human Body"; the *Fabrica*), challenging and correcting 16th century understanding of normal human anatomy. Vesalius's successors (Colombo, Fallopius, and Eustachius) further improved the accuracy of human anatomic detail. Thus, correction of Galen's anatomical inaccuracies (including the rete mirabile at the base of the brain, blood vasculature, the five lobed liver, and curved humerus) required at least 13 centuries for challenge, scientific disproof, and eventual medical community acceptance.

The Scientific Revolution describes the progressive change in attitude of scientists and physicians toward understanding of the natural world, health, and disease. This revolution began circa 1543 CE, when Copernicus published arguments for a heliocentric universe and Vesalius published the *Fabrica* series on human anatomy. By the 17th century, Galileo, Kepler, Newton, Harvey, and others had used this observation-based, matter-based, and mathematical law-based perspective to develop a scientific approach similar to our own modern approach of testing hypotheses with experimental data and statistics. In human biology, the investigation of structure led to studies of function, initially of human cardiovascular physiology, for example, Harvey's *Anatomical Exercise Concerning the Motion of the Heart and Blood in Animals* (1628). Whereas Galen conceived of parallel but unconnected arteries and veins, with continuous blood production in the liver and continuous blood consumption in the periphery, Harvey demonstrated that blood was pumped by the heart through arteries, through tissue capillaries, to veins, and then back to the heart in a circle (circulation). Correction of these and other Galenic physiological inaccuracies (such as nasal secretions representing the filtrate of cerebral ventricle fluid) thus required at least 14 centuries before challenge, scientific disproof, and eventual medical community acceptance.

The scientific method facilitates empirical, rational, and skeptical approaches to observational data, and minimizes human dependence on non-evidence-based traditional models. In spite of the scientific method, physicians are still human, and the medical community still shows an inertial reluctance to adapt to new information when it disrupts traditional paradigms. Recent examples would include reluctance to accept an etiologic role for the *H. pylori* bacterium in peptic ulcer disease, and reluctance to offer less than radical mastectomy for primary breast carcinoma.

Discovery of the microscopic world

Before the use of lenses to magnify objects, it was physically impossible to make observations on objects smaller than the resolving limit of the human eye (about 0.1−0.25 mm). Thus, prokaryotic and eukaryotic cells, tissue architecture, and comparisons of normal and disease microanatomy were philosophical speculation until description of the mathematics of optics and lens design. In a real sense, optical technology was rate-limiting for the development of the fields of tissue anatomy, cellular biology, and microbiology. Concepts of optics were written as early as 300 BCE in Alexandria (*Optica*, Euclid). Clear glass (crystallo) was developed in Venice in the 15th century. A compound microscope was invented by Janssen in 1590 CE. Microanatomy and structural terminology was begun by Malpighi (1661 CE), who examined capillaries in frog lung, trachea tubes for airflow in silkworms, and stomata in plant leaves. Robert Hooke used a compound microscope to describe common objects in *Micrographia* (1665 CE). Antony Van Leeuwenhoek used self-made simple magnifying lenses to count the threads in cloth in a Dutch dry-goods store, then later published descriptions of bacteria (termed *animalcules*), yeast, and algae, beginning in 1673 CE. Yet the relevance of these observations in microanatomy and microbiology to human diseases required changes in conceptual understanding of the etiology and pathogenesis of diseases. For example, it was 200 years after Van Leeuwenhoek that a *Streptococcus* sp. was recognized by Koch and Pasteur as the likely etiologic agent of puerperal fever in post-partum women.

Critical developments during the 19th century

Cellular pathology, germ theory, and infectious etiologic agents

The relevance of microanatomy and microbiology to human disease required expansion of conceptual understanding to include morphologic changes in diseased cells and tissues, as well as recognition of an etiologic role for microorganisms. Rokitansky's gross correlates with clinical disease (*A Manual of Pathological Anatomy*, 1846), Paget's surgical perspective on gross pathology, and Virchow's morphologic correlates with clinical disease were critical to the development of clinico-pathologic correlation, and served to create a role for pathologists to specialize in autopsy and tissue diagnosis. Virchow's description of necrotizing granulomatous inflammation, the morphologic correlate of infections caused by mycobacteria such as TB and leprosy, preceded the discovery of the etiologic agents years later by Hansen (*M. leprae*, 1873) and Koch (*M. tuberculosis*, 1882).

The causal relationship between microorganisms and clinical disease required scientific demonstration and logical proof before medical community acceptance. For example, identification of the cause-and-effect relationship between

Streptococcus sp. and puerperal fever required an initial recognition of unusual clinical outcomes (clusters of post-partum deaths), then correlation of puerperal fever clusters with obstetrician habits, then Semmelweiss's experimental demonstration in 1847 that hand-washing reduced the incidence of puerperal fever, then the demonstration that particular bacteria (*Streptococci*) are regularly associated with the clinical disease (Koch, circa 1870), and finally by culture of the organism from the blood of patients with the disease (Pasteur, 1879).

'Germ theory' articulates this causal relationship between microorganisms and clinical diseases in animals and humans. Technical improvements in microscopes (Abbe condenser, apochromatic lenses, oil immersion lenses), the development of culture media, and the development of histochemical stains no doubt made it possible for Koch to identify *M. tuberculosis* in 1882. To be emphasized is the process of recognition, first of all of the variables associated with the clinical disease, then the scientific demonstration of a causal relationship between one or more of these variables with the clinical disease. This latter step was enunciated as Koch's postulates (1890): (i) the bacteria must be present in every case of the disease, (ii) the bacteria must be isolated from a diseased individual and grown in pure culture, (iii) the specific disease must be reproduced when a pure culture is inoculated into a healthy susceptible host, and (iv) the same bacteria must be recoverable from the experimentally infected host.

Viruses, like bacteria, were understood and manipulated clinically prior to their isolation. Manipulation of viruses as vaccines is traced to description of smallpox variolation in Turkey by at least 1718 (described by Lady Montagu). Smallpox variolation was used in the US continental army in the 1770s. Local availability of cowpox, a non-lethal poxvirus, allowed Jenner to demonstrate (cross-) vaccination against smallpox (1796). Vaccination against smallpox was used in Napolean's army in 1812, and was mandated for Massachusetts schoolchildren by 1855. The success of these vaccination programs prompted development of vaccines against other human viruses through the 19th and 20th centuries, including rabies (Pasteur, 1885), yellow fever virus (Theiler, 1936), influenza A/B (1942), polio virus (Salk 1955, Sabin 1960), rubeola (1962), rubella (1969), hepatitis B virus (1981), varicella/zoster (1981). Transmission electron microscopy (Ruska, Knoll, 1931) allowed visualization of viruses, and large-scale sequencing starting in the 1990s allowed publication of viral genome sequences. Vaccination efforts are one of the great public health success stories of human history, and have reduced (rubella, rubeola, mumps, influenza, polio) or eliminated (smallpox, 1980) several of these viruses worldwide. Rapid identification of viruses in infected individuals is now possible, e.g. influenza virus ID by reverse-transcriptase PCR detection of type-specific RNA, or by immunosassay detection of type-specific proteins.

Organic chemistry

Prior to 1828, organic (carbon-containing) compounds were thought to derive from living organisms, and it was thought that they could never be synthesized from nonliving (inorganic) material. This concept ('vitalism') was disproven by the *in vitro* synthesis of urea by F. Wohler in 1828. Work from this era initiated the field of organic chemistry. Predictable rules for *in vitro* and *in vivo* organic reactions, structural theory, modeling, separation technologies, and accurate measurement subsequently allowed the chemical description of natural products, and the chemical synthesis of both natural products and synthetic compounds. In addition to setting the stage for a systematic understanding of cellular biochemistry and physiology, organic chemistry set the stage for laboratory synthesis of natural products, such as dyes, vitamins, hormones, proteins, and nucleic acids. In that era, the textile industry was the main consumer of dyes. Access to imported natural product dyes from plants was predictable for seafaring nations, but not for landlocked nations. In parallel with natural product extraction and purification was the recognition that aniline from coal (Perkin, 1856) could be modified to generate a spectrum of colors, catalyzing the development of the German dye industry in the last half of the 19th century. Some of these synthetic dyes were found useful for histochemical staining.

Histotechnology

Morphologic diagnosis requires thin ($3-5$ μm), contrast-rich (requiring dyes) sections of chemically fixed (cross-linked or precipitated) tissue. Thin sections allow the passage of light through the tissue, but reduce overlapping of cells in the light path. Thus, technologies had to develop for cutting and staining of thin fixed tissue sections. Work leading to our current technique for tissue solidification in paraffin wax was first described by Klebs in 1869. Prototypes of our current mechanical microtome for making thin (~5 μm thick) tissue sections was developed by Minot in 1885. Precursor work leading to our current technique for tissue fixation with diluted formalin was first described by Blum in 1893.

Histochemical stains developed in parallel with dye technology for the textile industry. Botanists used carmine as a stain in 1849, and subsequently Gerlack applied carmine to stain brain tissue in 1858. The current hematoxylin dye used in tissue histochemistry was originally extracted from logwood trees from Central America for the dye industry (to compete with indigo). Metallic ion mordants made oxidized hematoxylin (hematein) colorfast in textiles, and a protocol

for tissue staining was published by Boehmer in 1865. Similar to the hematoxylin story, semisynthetic analine dye technology was adapted by histochemists from 1850 to 1900. Many of these dyes are still routinely used for recognition of tissue structure, peripheral blood cells, and microorganisms, including hematoxylin, eosin, methylene blue, Ziehl-Neelsen, Gram, van Gieson, Mallory trichrome, and Congo red.

The most commonly used stains for general tissue diagnosis are the hematoxylin and eosin (H&E) stains, which provide a wealth of nuclear and cytoplasmic detail not visible in an unstained section. Supplemental histochemical stains demonstrate specific structures and organisms: collagen and muscle (trichome), elastin (Verhoff-von Giessen), glycogen/mucin (periodic acid-Schiff, PAS), mucin (PAS diastase, mucicarmine, alcian blue), fungi (Gemori methenamine silver, GMS), mycobacteria (Ziehl-Neelsen, Fite), and bacteria (Gram, Warthin-Starry). Each of these stains is inexpensive ($10−$50), fast (minutes to hours), and automatable, making them extremely valuable for service diagnostic pathology use.

Light microscopy lens technology matured during the last half of the 19th century. Critical were Abbe's introductions of the apochromatic lens system to eliminate chromatic aberration (different focal lengths for different wavelengths of visible light) in 1868, a novel condenser for compound microscopes (to provide better illumination at high magnification) in 1870, and an oil immersion lens in 1878.

By 1900, maturation of tissue fixation chemistry, histochemical stain protocols, and light microscope technology had evolved into the workhorse technique for evaluation of morphologic abnormalities in tissue examination in anatomic pathology labs, and for the evaluation of morphologic features of microorganisms in microbiology labs. The scope of this chapter is limited to pathology, but it should be clear to the reader that the momentous discoveries of deep general anesthetics (Long, 1841; Morton, 1846), commercial electricity (Edison, 1882), and radiography (Roentgen, 1895) also contributed to the development of the modern medical specialties of diagnostic pathology and laboratory medicine.

Developments during the 20th century

Humoral and cellular immunology

The development of antisera in the 20th century for therapeutic purposes (for instance, treatment of diptheria) led to progressive understanding of the antibody, the efferent arm of the humoral immune response. Similarly, tissue transplantation experiments led to the recognition of cellular rejection due to thymus-derived T-cells. Antibodies and T-cells cooperate to react to foreign (non-self) molecules, common examples being allergic responses, viral infections, and organ transplants. Antibodies were found to be made by B-cells and plasma cells, and were found to be exquisitely specific for binding to their particular antigens (ligands) either in solid phase or in solution. The analogous T-cell receptor (TCR) recognizes a ligand made up of a 15−20mer peptide presented by self MHC (HLA in human) molecules on the surface of antigen-presenting cells (macrophages, dendritic cells, activated B-cells). B-cell and T-cells activate and proliferate when exposed to non-self proteins, but not to self proteins, attesting to tolerance to self. When B-cell and T-cell self-tolerance breaks down, autoimmune diseases can result (including myasthenia gravis, Grave's disease, and lupus erythematosus). Antibodies (immunoglobulins) were found to be heterodimers of 50 kD heavy chains and 25 kD light chains, folded together so that a highly variable portion defines the antigen-binding site, and a constant portion defines the isotype (IgM, IgD, IgG, IgE, or IgA). Similarly, T-cell receptors were found to be heterodimers of immunoglobulin-like molecules with a highly variable portion for ligand binding, and a constant portion, but without different isotypes. The range of antigen-binding specificities in a normal mammal is extensive, perhaps infinite, subtracting out only those self proteins to which the animal is tolerant. The genes encoding immunoglobulins and T-cell receptors were sequenced and, surprisingly, the extensive variation of specificities was due to a unique system of gene rearrangements of polymorphic V, D, and J gene segments with random nucleotide addition at the junctions. This system allows generation of literally billions of different Ig and TCR binding specificities. Although the Ig and TCR molecules define the specificity of antigen (ligand) binding, we now understand that the probability of T lymphocyte activation is adjustable, based on activating (e.g. CD28:B7) or inhibitory (e.g. PD-1:PD-L1) receptor:ligand interactions.

Polyclonal antibodies raised in other species (goat, mouse, rabbit) against an antigen can be used to detect the antigen in diffusion gels (Ochterlony, western blot), in solution (ELISA), and in tissue sections. Use of fluorescent-tagged antibodies for frozen section immunohistochemistry was developed first, and immunofluorescence (IF) is still routinely used in renal pathology and dermatopathology. Peroxidase-tagged secondary antibodies and DAB chemistry were developed to generate a stable chromogen in the tissue, and this is now the primary method for detecting antigens in formalin-fixed tissue sections. Improved antibody binding specificity and industrial production required monoclonal

antibodies, which in turn required the development of mouse plasmacytomas/myelomas and cell fusion protocols. The net result is that commercial antibodies are now available for antigens of both clinical and research interest, including antibodies specific enough to distinguish minimally modified variant antigens (for instance, phosphorylated proteins or proteins with single amino acid substitutions).

Natural product chemistry and the rise of clinical laboratories

Diseases due to dietary deficiencies (like scurvy) and hormonal imbalances (like diabetes mellitus) were described clinically long before they were understood pathologically. Dietary deficiency diseases prompted searches for the critical metabolic cofactors, so-called 'vital amines' (vitamins). Xerophthalmia was linked to retinol (vitamin A) deficiency in 1917 (McCollum). Rickets was linked to calcitriol (vitamin D) deficiency in 1926. Beri-beri was linked to a deficiency of thiamine (vitamin B1) in 1926. Scurvy was linked to ascorbic acid (vitamin C) deficiency in 1927. Pellagra in the United States was linked to niacin deficiency in 1937. Pernicious anemia was linked to cobalamin (vitamin B12) deficiency in 1948. It is currently unusual to see morphologic features of these diseases in this country.

Diseases due to non-dietary physiologic imbalances prompted isolation of circulating molecules with systemic effects, i.e. the hormones. Parathyroid hormone was isolated (Berman, Collip) in the 1920s. Thyroxine and cortisone were isolated (Kendall) in 1915, and table salt was iodized starting in 1917. Insulin was isolated (Banting, Best) in 1921, non-human insulin was industrially purified and marketed soon thereafter, and recombinant human insulin was marketed starting in 1982.

These examples highlight the ability of 20th century chemists to fractionate, purify, synthesize, measure bioactivity, and manufacture these compounds for safe use by humans. Study of diseases due to deficiencies and excesses of single molecule function led to a mechanistic understanding of biochemistry and physiology, with resultant interconnected reaction pathways of byzantine complexity (now referred to as systems biology). Clinical demand for body fluid levels of ions (such as sodium, potassium chloride, and bicarbonate), glucose, creatinine, hormones (such as thyroxine and parathyroid hormone), albumin, enzymes (related to liver and cardiac function), and antibodies (reactive to ASO, Rh, ABO, and HLA antigens) led to the development of clinical laboratories in chemistry, endocrinology, immunopathology, and blood banking. Functional assays for coagulation cascade status were developed, as were methods for estimating blood cell concentration and differential, leading to coagulation and hematology laboratories. Serologic and cell activation assays to define HLA haplotype led to HLA laboratories screening donors and recipients in anticipation of bone marrow and solid organ transplants. Culture medium-based screening for infectious agents led to dedicated clinical microbiology laboratories, which are beginning to incorporate nucleic acid screening technologies for speciation and prediction of treatment response. The clinical laboratories now play a critical, specialized role in inpatient and outpatient management, and their high test volumes (a 700-bed hospital may perform 5 million tests per year) have catalyzed computer databases for central record-keeping of results.

Natural product chemistry: nucleic acids

The previous vignettes indicate a scientific approach to natural products of the steroid and protein types, but do not indicate how proteins are encoded, what accounts for variation in the same protein in the population, or how inherited diseases are inherited. It turns out that the instruction set for protein sequence is defined by DNA sequence. The role of nucleoproteins as a genetic substance was alluded to by Miescher in 1871, and was shown by Avery to be the pneumococcal transforming principle in 1944. The discovery of X-ray crystallography in 1912 made it possible for Franklin, Wilkins, and Gosling to study DNA crystal structure, and led to the description of the antiparallel double helix of DNA by Watson and Crick in 1953. This seminal event in history facilitated dissection of the instruction set for an organism, with recognition that 3-base codons specified amino acids in 1961, and description of the particular codons encoding each amino acid in 1966. Demonstration of *in situ* hybridization in 1969 made it possible to localize specific DNA or RNA sequences within the cells of interest. Recognition and purification of restriction endonucleases and DNA ligases, and the development of cloning vectors, made it possible to clone individual sequences, leading to methods for synthesis of natural products (such as recombinant human insulin in 1978). Chemical methods were developed for sequencing DNA, initially with radioisotope-tagged nucleotide detection in plate gels, then with fluorescent nucleotide detection in 1986. Subsequent conversion to capillary electrophoresis and computer scoring of sequence output allowed high throughput protocols which generated the human genome sequence by 2001. The development of polymerase chain reaction (PCR) chemistry in 1986 has made it possible to quickly screen for length polymorphisms of microsatellite (identity testing, donor:recipient ratio after bone marrow transplant, and microsatellite instability), coding sequence abnormalities in genes (translocations, rearrangements, insertions, deletions, substitutions), and epigenetic control of

transcription (promoter methylation) in a targeted fashion. Quantitative PCR methods using fluorescent detection of amplicons has made it possible to study DNA and RNA copy number, and to mimic Northern blots/oligo microarrays in estimating RNA transcript abundance for cluster analysis. Massively parallel solid-state ("next-generation") sequencing chemistries now allow rapid alignment of hundreds of independent sequencing reactions, facilitating rapid comparison of germline and somatic sequence, as well as identification of low-abundance mutations.

Current practice of pathology

Diseases can be distinguished from each other based on differences at the molecular, cellular, tissue, fluid chemistry, and/or individual organism level. One hundred and sixty years of attention to the morphologic and clinical correlates of diseases has led to sets of diagnostic criteria for the recognized diseases, as well as a reproducible nomenclature for rapid description of the changes associated with newly discovered diseases. Sets of genotypic and phenotypic abnormalities in the patient are used to determine a diagnosis, which then implies a predictable natural history and can be used to optimize therapy by comparison of outcomes among similarly afflicted individuals. The disease diagnosis becomes the management variable in clinical medicine, and management of the clinical manifestations of diseases is the basis for day-to-day activities in clinics and hospitals nationwide. The pathologist is responsible for integration of the data obtained at the clinical, gross, morphologic, and molecular levels, and for issuing a clear and logical statement of diagnosis.

Clinically, diseases present to front-line physicians as patients with sets of signs and symptoms. Symptoms are the patient's complaints of perceived abnormalities. Signs are detected by examination of the patient. The clinical team, including the pathologist, will work up the patient based on the possible causes of the signs and symptoms (the differential diagnosis). Depending on the differential diagnosis, the workup typically involves history-taking, physical examination, radiographic examination, fluid tests (blood, urine, sputum, stool), and possibly tissue biopsy.

Radiographically, abnormalities in abundance, density or chemical microenvironment of tissues allow distinction from surrounding normal tissues. Traditionally, the absorption of electromagnetic waves by tissues led to summation differences in exposure of silver salt photographic film. Tomographic approaches such as computerized tomography (CT, 1972) and nuclear magnetic resonance (NMR, MRI, 1973) complemented summation radiology, allowing finely detailed visualization of internal anatomy in any plane of section. In the same era, ultrasound technology allowed visualization of tissue with density differences, such as a developing fetus or gallbladder stones. More recently, physiology of neoplasms can be screened with positron emission tomography (PET, 1977) for decay of short half-life isotopes such as fluorodeoxyglucose. Neoplasms with high metabolism can be distinguished physiologically from adjacent low-metabolism tissues, and can be localized with respect to normal tissues by pairing PET with standard CT. The result is an astonishingly useful means of identifying and localizing new space-occupying masses, assigning a risk for malignant behavior and, if malignant, screening for metastases in distant sites. This technique is revolutionizing the preoperative decision-making of clinical teams, and improves the likelihood that patients undergo resections of new mass lesions only when at risk for morbidity from malignant behavior or interference with normal function.

Pathologically, disease is diagnosed by determining whether the morphologic features match the set of diagnostic criteria previously described for each disease. Multivolume texts are devoted to the gross and microscopic diagnostic criteria used for diagnosis, prognosis, and prediction of response to therapy. Pathologists diagnose disease by generating a differential diagnosis, then finding the best fit for the clinical presentation, the radiographic appearance, and the pathologic (both clinical lab and morphologic) findings. Logically, the Venn diagram of the clinical, radiologic, and pathologic differential diagnoses should overlap. Unexpected features expand the differential diagnosis and may raise the possibility of previously undescribed diseases. For example, Legionnaire's disease, human immunodeficiency virus (HIV), Hantavirus pneumonia, and severe acute respiratory syndrome (SARS) are examples of newly described diagnoses during the last 40 years. The mental construct of etiology (cause), pathogenesis (progression), natural history (clinical outcome), and response to therapy is the standard approach for pathologists thinking about a disease. A disease may have one or more etiologies (initial causes, including agents, toxins, mutagens, drugs, allergens, trauma, or genetic mutations). A disease is expected to follow a particular series of events in its development (pathogenesis), and to follow a particular clinical course (natural history). Disease can result in a temporary or lasting change in normal function, including patient death. Multiple diseases of different etiologies can affect a single organ, for example, infectious and neoplastic diseases involving the lung. Different diseases can derive from a single etiology, for example, emphysema, chronic bronchitis, and small cell lung carcinoma in long-term smokers. The same disease (for instance, emphysema of the lung) can derive from different etiologies (emphysema from α-1-antitrypsin deficiency or cigarette smoke).

Modern surgical pathology practice hinges on morphologic diagnosis, supplemented by special stains, immunohistochemical stains, cytogenetic/molecular data, and other clinical laboratory findings, as well as on the clinical and radiographic findings. Findings that meet all of these criteria are diagnostic for the disease. If some, but not all, of the criteria are present to make a definitive diagnosis, the pathologist must either equivocate or make an alternate diagnosis. Thus, a firm grasp of the diagnostic criteria, and the instincts to rapidly create and sort through the differential diagnosis, must be possessed by the service pathologist.

The tissue diagnosis has to make sense, not only from the morphologic perspective, but from the clinical and radiographic vantage points as well. It is both legally risky and professionally erosive to make a clinically and pathologically impossible diagnosis. In the recent past, limited computer networking meant numerous phone calls to gather the relevant clinical and radiographic information to make an informed morphologic diagnosis. For example, certain diseases such as squamous and small cell carcinomas of the lung are extremely rare in non-smokers. Thus, a small cell carcinoma in the lung of a non-smoker merits screening for a non-pulmonary primary site. Fortunately for pathologists, computing and networking technologies now allow access to preoperative clinical workups, radiographs/reports, clinical laboratory data, and prior pathology reports. All of these data protect pathologists by providing them with the relevant clinical and radiographic information, and protect patients by improving diagnostic accuracy. Just as research scientists "*… ignore the literature at their peril …*", diagnostic pathologists "*… ignore the presentation, past history, workup, prior biopsies, and radiographs at their peril…*"

There are limitations to morphologic diagnosis by H&E stains. First, lineage of certain classes of neoplasms (including small round blue cell tumors, clear cell neoplasms, spindle cell neoplasms, and undifferentiated malignant neoplasms) is usually clarified by immunohistochemistry, frequently by cytogenetics (when performed), and sometimes by electron microscopy. Second, there are limitations inherent in a snapshot biopsy or resection. Thus, the etiology and pathogenesis can be obscure or indeterminate, and rates of growth, invasion, or timing of metastasis cannot be inferred. Third, the morphologic changes may not be specific for the underlying molecular abnormalities, particularly the rate-limiting (therapeutic target) step in the pathogenesis of a neoplasm. For example, *RET* gain of function mutations in a medullary thyroid carcinoma will require DNA level screening to determine germline involvement, familial risk, and presence or absence of a therapeutic target. Fourth, the same morphologic appearance may be identical for two different diseases, each of which would be treated differently. For example, there is no morphologic evidence by H&E stain alone to distinguish host lymphoid response to Hepatitis C viral (HCV) antigens from host lymphoid response to allo-HLA antigens in a liver allograft. This is obviously a major diagnostic challenge when the transplant was done for HCV-related cirrhosis, and when the probability of recurrent HCV infection in the liver allograft is high.

Paraffin section immunohistochemistry has proven invaluable in neoplasm diagnosis for clarifying lineage, improving diagnostic accuracy, and guiding customized therapy. If neoplasms are poorly differentiated or undifferentiated, the lineage of the neoplasm may not be clear. For example, sheets of undifferentiated malignant neoplasm with prominent nucleoli could represent carcinoma, lymphoma, or melanoma. To clarify lineage, a panel of immunostains is performed for proteins that are expressed in some of the neoplasms, but not in others. Relative probabilities are then used to lend support (rule in) or exclude (rule out) particular diagnoses in the differential diagnosis of these several morphologically similar undifferentiated neoplasms. The second role is to make critical distinctions in diagnosis that cannot be accurately made by H&E alone. Examples of this would include demonstration of myoepithelial cell loss in invasive breast carcinoma but not in its mimic, sclerosing adenosis (Fig. 11.2), or demonstration of loss of basal cells in invasive
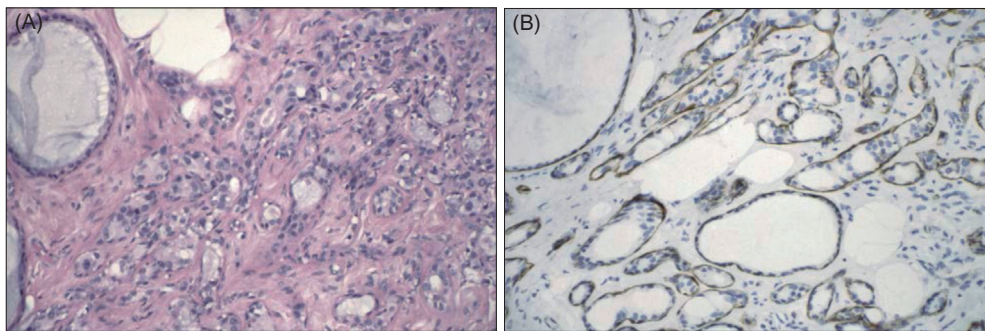

FIGURE 11.2   Sclerosing adenosis of breast. (A) Hematoxylin and eosin (H&E) staining of sclerosing adenosis of breast. By H&E alone, the differential diagnosis includes infiltrating ductal carcinoma and sclerosing adenosis. (B) Actin immunostain of sclerosing adenosis of breast. Actin immunoreactivity around the tubules of interest supports a diagnosis of sclerosing adenosis and serves to exclude infiltrating carcinoma.

prostatic adenocarcinoma (Fig. 11.3). The third role of immunohistochemistry is to identify particular proteins, such as nuclear estrogen receptor (ER) (Fig. 11.4) or the plasma membrane HER2 proteins (Fig. 11.5), both of which can be targeted with inhibitors rather than generalized systemic chemotherapy. Morphology remains the gold standard in this diagnostic process, such that immunohistochemical data support or fail to support the H&E findings, not vice versa.
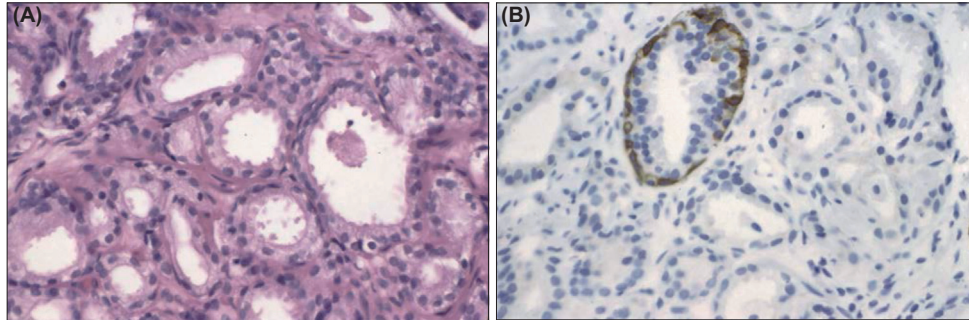

FIGURE 11.3 Invasive adenocarcinoma of prostate. (A) Hematoxylin and eosin (H&E) staining of invasive adenocarcinoma of prostate. By H&E alone, the differential diagnosis includes invasive adenocarcinoma and adenosis. (B) High molecular weight cytokeratin immunostain of invasive adenocarcinoma of prostate. Loss of high molecular weight cytokeratin (34βE12) immunoreactivity around the glands of interest supports a diagnosis of invasive adenocarcinoma.
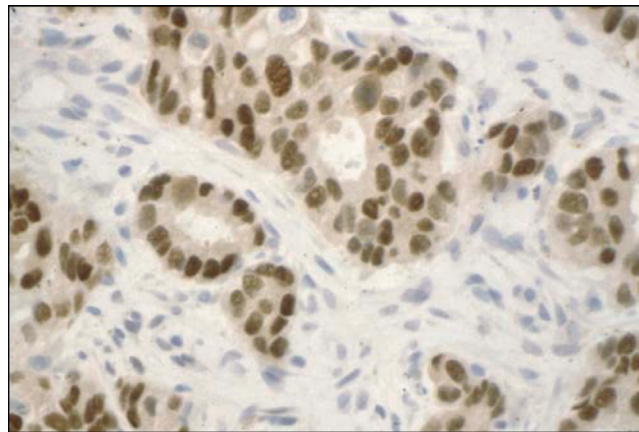

FIGURE 11.4 Estrogen receptor immunostain of breast carcinoma. Strong nuclear immunoreactivity for ER is noted, guiding use of ER inhibitor therapy.
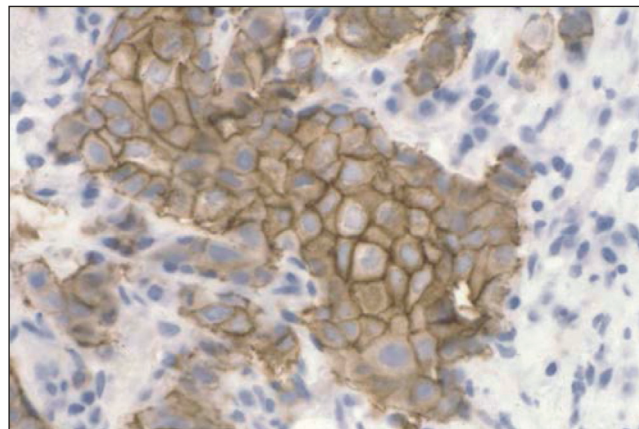

FIGURE 11.5 HER2/c-erbB2 immunostain of breast carcinoma. Strong plasma membrane immunoreactivity for c-erbB2/HER2 is noted, guiding use of either anti-HER2 antibody or HER2 kinase inhibitor therapy.

Probability and statistics are regular considerations in immunohistochemical interpretation, since very few antigens are tissue-specific or lineage-specific. Cytokeratin is positive in carcinomas, but also in synovial and epithelioid sarcomas. This example may imply aspects of the lineage of these two sarcomas that may be helpful in our categorization of these neoplasms. Another example would be the diagnosis of small cell carcinoma in the lung of a nonsmoker. Because lung primary small cell carcinoma is extremely uncommon, in non-smokers, this diagnosis would prompt the pathologist to inquire about screening results for other, non-pulmonary, sites. Likewise, immunohistochemistry results are always put into the context of the morphologic, clinical, and radiographic findings. For example, an undifferentiated CD30+ neoplasm of the testis supports embryonal carcinoma primary in the testis, whereas a lymph node effaced by sclerotic bands with admixed CD30+ Reed-Sternberg cells supports nodular sclerosing Hodgkin's disease.

Demand for both diagnostic accuracy and report promptness has increased as hospitals come under increasing financial pressure to minimize length of patient stay. Hospitals now manage all but the sickest patients as outpatients. Minimally invasive approaches for the acquisition of tissue samples for diagnosis use flexible endoscopic biotomes or hollow needles that sample $1-2$ mm diameter tissue specimens. Multidisciplinary conferences function almost real-time with respect to the initial biopsies. Together, these changes have forced modern pathologists to make critical diagnoses on progressively smaller biopsy specimens, sometimes bordering on the amounts seen in cytopathology aspirates, and to do this in a timely fashion. This requires a clear understanding of the limitations to development of an accurate diagnosis, and a willingness on the part of the pathologist to request repeat biopsy for additional tissue when it is necessary for accurate diagnosis.

Diagnostic criteria involving electron microscopic ultrastructure found relevance for the evaluation of neoplasms described as small round blue cell tumors, spindle cell tumors, melanocytic tumors, and neuroendocrine/neuroblastic tumors, as well as delineation of ciliary ultrastructural abnormalities in primary ciliary dyskinesia. Current approaches to these neoplasms are now generally approached using paraffin section immunohistochemistry. Electron microscopy is now currently used mainly for nephropathology, platelet morphology, ciliary axoneme morphology, and for rare cases where immunohistochemistry is not diagnostic and where demonstration of premelanosomes, neuroendocrine granules, or amyloid is diagnostic.

Adequate sampling of a lesion is critical to making an accurate diagnosis. Undercall diagnostic discrepancies are frequently due to sampling of a small portion of a large lesion that is not representative of the most abnormal portion of the lesion. Insufficient sampling can result in an equivocal diagnosis or, worse, an inaccurate diagnosis. Empirical rules have been adopted over the decades to ensure statistically adequate sampling of masses and organs, e.g. transurethral resections of prostate, soft tissue sarcomas, and heart allograft biopsies.

In spite of the limitations and statistical uncertainties relating to morphologic diagnosis, a wealth of information is conveyed to a service pathologist in a tried-and-true H&E section. Analogous to the fact that a plain chest X-ray is the sum total of all densities in the beam path, the morphologic changes in diseased cells and tissues are the morphologic sum total of all of the disequilibria in the abnormal cells. For most neoplastic diseases, morphologic criteria are sufficient to predict the risk of invasion and metastasis (the malignant potential), the pattern of metastases, and the likely clinical outcomes. For example, the etiology and pathogenesis in small cell lung carcinoma can be inferred (cigarette smoking, with carcinogen-induced genetic mutations) and the outcome predicted (early metastasis to regional nodes and distant organs, with high probability of death within 5 years of diagnosis). New molecular data for both neoplastic and non-neoplastic diseases will most likely benefit unaffected individuals by estimating disease risk, and will most likely benefit patients by defining the molecular subset for morphologically defined diagnostic entities, thus guiding individualized therapy.

The future of diagnostic pathology

Diagnostic pathology will continue to use morphology and complementary data from protein (immunohistochemical) and nucleic acid (cytogenetic, *in situ* hybridization, DNA sequence, epigenetic, and RNA abundance) screening assays. Improvements in current technologies should improve current test performance, reduce test cost, and shorten turnaround time. Development of new technologies will lead to better diagnostic algorithms, improved diagnostic accuracy and prognosis, improved patient assignment to prospective randomized clinical trials, improved prediction of response to customized therapy, and improved sensitivity for measurement of residual subclinical disease.

Individual identity at the molecular level

For transplant candidates, major histocompatability complex (MHC, HLA in human) screening is evolving from cellular assays and serology toward sequencing of the alleles of the class I and II HLA loci. Rapid sequencing of these alleles

in newborn cord blood would allow creation of a database of the population's haplotypes, facilitating perfect matches for required bone marrow or solid organ transplants.

Rapid cytogenetics

Current uses of *in situ* hybridization to screen for viruses (such as EBV), light chain restriction (in B lymphomas), and copy number variation (for instance, HER2 gene amplification) demonstrate the benefit of *in situ* nucleic acid hybridization assays. Interphase *in situ* hybridization with fluorescent probes (FISH) is now being used in the initial diagnostic workup for certain diseases, e.g. sarcoma-specific translocations, ploidy analysis in hydatidiform moles, and copy number variation analysis for detection of locus amplification and deletion.

Rapid DNA sequence, RNA sequence, and RNA abundance screening

Current uses of DNA screening for *BCR-ABL* translocation, donor:recipient ratios after allogeneic bone marrow transplant, microsatellite instability, quantitative viral load (e.g. EBV, BK, CMV), single gene mutations (e.g. *CFTR*, *Factor 2*, *SERPINA1* (α-1-antitrypsin), *HFE*), and promoter methylation (e.g. *MLH1*, *MGMT*) demonstrate the benefit of nucleic acid screening in diagnosis and management. It is likely that each new malignant neoplasm will be promptly inventoried for chromosomal ploidy, gene translocations, gene copy number variation, DNA base substitutions/insertions/deletions, annotation of non-synonymous coding sequence and splice-site changes as either benign polymorphisms or pathogenic mutations, local and global promoter methylation status, and RNA expression cluster subset. This inventory at the time of initial diagnosis, prior to therapy, should allow molecular subgrouping, individualized therapy, and targeted residual disease screening. As the cost of genome sequencing approaches the $1000 mark, sequencing of newborns for germline mutations that predispose to subsequent developmental or neoplastic diseases now seems practical for the purposes of early diagnosis, prompt management, and routine follow-up.

Computer-based prognosis and prediction

Current uses of morphology, immunohistochemistry, and molecular pathology demonstrate their benefit through improved diagnostic accuracy. However, diagnosis, extent of disease, and molecular subsets are currently imperfect estimators of prognosis and response to therapy. Relational databases which correlate an individual's demographic data, family history, and concurrent diseases with the neoplasm's morphologic features, immunophenotype, and molecular subset, and which integrate disease prevalence by age, sex, and ethnicity using Bayesian probabilities, should improve accuracy of prognosis and prediction of response to therapy. As risk correlates are developed, it is possible that healthy individuals can be screened and given risk estimates for development of different diseases, as desired. It is worth noting here that unaffected patients in inherited-disease kindreds such as Huntington's disease may prefer not to know what the future holds with respect to the disease that runs in their extended family, so germline screening should hinge on pre test patient consent.

Normal ranges and disease risks by ethnic group

Current uses of normal ranges for serum chemistry assume similar bell-curve distributions across ages, sexes, and ethnicity. This may not be true for all analytes, so normal ranges should be measured and compared, with publication of analytes whose normal ranges differ by age, sex, or ethnic group. Computer reference databases facilitate this sort of normal range database, stratified by age/sex/ethnicity of individual patients. Similarly, familial risk for an inherited disease may vary by ethnic group, and this variation may be used in Bayesian calculations to define risk for unaffected at-risk family members.

Individual metabolic differences relevant to drug metabolism

Current uses of liver and renal impairment to guide drug dosage demonstrate the benefit of using patient physiology to customize therapy. We are now starting to use gene haplotype data for specific genes that encode specific drug-metabolizing enzymes, so as to guide starting doses for particular drugs (e.g. warfarin, tamoxifen, or clopidogrel/Plavix) prior to treatment initiation.

Serum biomarkers and residual disease testing

Current uses of prostate specific antigen (PSA) to screen for prostate carcinoma and its recurrence demonstrate the benefit of serum biomarkers in common neoplasms. It is likely that high-sensitivity screening for single and clustered serum analytes using proteomic and metabolomic technologies will lead to improved methods for early detection of neoplasms, autoimmune diseases, and infections. Residual disease testing for malignancies with clonal mutations or translocations can now be performed via assays based on circulating tumor cells or circulating cell-free tumor DNA (so-called liquid biopsies). Similar high-sensitivity testing for disease-specific analytes may also improve diagnosis, treatment, and residual disease testing for autoimmune diseases, and infections.

Treatment by pathway, rather than by tissue diagnosis

Traditional medical oncology chemotherapy strategies for malignant neoplasms hinged on the tissue diagnosis and the organ of origin. This is changing, as current knowledge of signaling pathway disruption by gain-of-function mutations, e.g. EGFR or BRAF, points to specific treatment targets. The pharmaceutical industry is able to create small-molecule inhibitors and humanized antibodies that allow specific treatment of specific mutant enzyme isoforms. Current data suggests that many, if not most, patients with driver gain-of-function mutations treated with specific inhibitors will show an initial brisk response, although later recurrence is possible due to outgrowth of subclones with different driver mutations. The challenge for the next generation of scientists and oncologists is how to rescue homozygous loss-of-function mutations that are etiologic in human diseases, e.g. *CFTR* or *P53*.

Conclusions

Pathologists consider each disease to have a natural, mechanical, physicochemical basis. Each disease has an etiology (initial cause), a pathogenesis (stepwise progression), and a natural history with effects on normal function (clinical outcome). Pathologists collect the data needed to answer patients' and clinicians' questions, simply phrased as "what is it?" (diagnosis), "how it going to behave?" (prognosis), and "how do I treat it?" (prediction of response to therapy). Instincts and diagnostic criteria, as well as the optical, mechanical, chemical, and computing technologies described previously, are the basis for modern service pathology. As the human genome is deciphered, and as the complex interactions of cellular biochemistry are refined, risk of disease in unaffected individuals will be calculable, disease diagnosis will be increasingly accurate and prognostic, and molecular subsets of morphologically defined disease entities will be used to guide customized therapy for individual patients. It is a great time in history to be a pathologist.

Key concepts

- Clinically, diseases present to front-line physicians as patients with sets of signs and symptoms. Symptoms are the patient's complaints of perceived abnormalities. Signs are detected by examination of the patient. The clinical team (including the pathologist) evaluate the patient based on the possible causes of the signs and symptoms (the differential diagnosis).
- Pathologically, disease is diagnosed by determining whether the morphologic features match the
- set of diagnostic criteria previously described for each disease. Pathologists diagnose disease by generating a differential diagnosis, then finding the best fit for the clinical presentation, the radiographic appearance, and the pathologic (both clinical lab and morphologic) findings.
- Etiology describes the causes of a disease. One disease entity can have more than one etiology, and a single etiology can lead to more than one disease. For example, emphysema, chronic bronchitis, and small cell lung carcinoma can all occur in long-term smokers (different diseases derived from a single etiology). Likewise, the same disease (for instance, emphysema of the lung) can derive from different etiologies (emphysema from a-1-antitrypsin deficiency or cigarette smoke).
- The pathogenesis of a disease describes its stepwise progression after initiation in response to a specific etiologic factor (or factors). Pathogenesis can refer to the changes in the structure or function of an organism at the gross/clinical level, and it can refer to the stepwise molecular abnormalities leading to changes in cellular and tissue function.
- The natural history of a disease describes the expected course of disease, including chronicity, functional impairment, and survival. However, not all patients with a given disease will naturally follow the same disease course, so differences in patient outcome do not necessarily correspond to incorrect diagnosis. Variables that

independently correlate with clinical outcome differences are called independent prognostic variables, and are assessed routinely in an effort to predict the natural history of the disease in the patient.
- Variables that independently correlate with response to therapy are called independent predictive variables, and are assessed routinely in an effort to optimize therapeutic response for each patient.

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