To Begoña, Pablo, Santiago, Maria, Laura, Lucas, and Cecilia
the reason for my existence and to Joel C. Eissenberg, who taught me
how to do science
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During the last few years there has been a great deal of public interest in the area of personalized medicine. News articles, scientific magazines, and entire books have been dedicated to the subject. While a lot has been said about the subject, there is little done in practice. Nowadays there are only a few examples of personalized medicine. One of them is the use of the HER2 diagnostic test, in breast cancer patients, in order to treat them with Herceptin, a drug that works well in that subpopulation. Other tests, like the estrogen receptor (ER) or the progesterone receptor (PR), are also used to put breast cancer patients in hormonal therapy. All these diagnostic tests could be characterized as molecular pathology tests.

My intent in putting this book together was to show others how one can develop new molecular pathology tests for use in personalized medicine. I have used the process of drug discovery and development as the outline of the book for a simple reason, the discovery of the molecular pathology test could be done at the same time that the drug is been discovered. In Chapter 1, Dr. Franz Fogt gives an overview and historical perspective of the field of molecular pathology, and I follow it with a simplified overview of the drug discovery and development process. Chapter 2 follows with a view of the drug discovery process and how molecular pathology could be used to identify and validate new drug candidates. Chapter 3 introduces the reader to the world of biomarkers, and how biomarkers could be found using transcriptional profiling. These biomarkers can then be used as surrogate endpoints, and molecular pathology could play a significant role in validating these biomarkers and developing tests for use in hospitals. This chapter is followed by examples of molecular pathology in safety assessment in the area of toxicology. It also gives an overview of toxicology and its methods to identify off-target liabilities of drugs in both small molecules and biological compounds. Chapter 5 looks at toxicogenomics, a new way of doing toxicology by looking at transcriptional profiling to identify genes that are relevant to the safety of compounds. This chapter is followed by the use of molecular pathology in clinical trials. Examples of how molecular pathology assays have helped identify the right dose for different drugs are shown. Not only is molecular pathology useful in finding the right dose but also in finding the right patients for treatment, which is discussed in Chapter 7. Here again is the area of personalized medicine that is directly affected by molecular pathology. Several examples are shown of how this is done today in the clinic. The following chapters deal more with direct
applications of molecular pathology. Chapter 8 shows several examples of usage of molecular pathology in molecular therapy. Chapter 9 is a practical approach on how to do immunohistochemistry (IHC), one of the most important and useful techniques in molecular pathology. This chapter also indicates if you do not have the expertise in house how to use other companies, contract research organizations, to do this type of work. The last two chapters look more at the future of molecular pathology. Chapter 10 deals with the quantification of the colorimetric signal while Chapter 11 looks at fluorescence as a way to quantify and normalize the signal.

Color representations of selected figures in the book are available as pdf files at the following ftp site address:


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1.1. GENERAL PATHOLOGY

The histopathologic assessment of tissues and, for that matter, body fluids serves to diagnose alterations and disease state and helps to categorize and collect information about disease. The pathologic assessment of tissues and organs itself is a stepwise process of progressive analysis of the present disease, and the next possible finding one can describe with (relative) certainty. This is, naturally, only possible when a sufficient amount of tissue is submitted to pathology. If fluid material, only cells present in that specimen can be assessed and further evaluation can mostly not be done with certainty. For the diagnosis of a colon carcinoma, a microscope is rarely necessary. When opened, the colon will reveal the tumor, the size, and, at least semiquantitatively, the invasive depth. However, to assess the correct depth and the type of carcinoma, a section of the tissue must be reviewed with the microscope. The next necessary diagnostic step to categorize, grade, and stage the lesion is the review of the lymph nodes as to their involvement by metastatic disease. Traditional histopathology uses the morphologic aspects of tissue and cellular arrangement to provide diagnosis as to the cellular origin of malignant tumors.

Similarly, morphologic features can be used to predict behavior and outcome of malignant tumors and can influence the way certain tumors are treated. This
is illustrated by the relative bland morphology of bronchioloalveolar carcinomas of the lung with a relatively benign outcome compared to the guarded outcome of poorly differentiated small-cell carcinomas of the same organ. In the case of colorectal carcinomas, the morphologic aspect of tumor transgressing through all layers of the bowel wall and its presence as metastatic tumor within lymph nodes indicates a higher stage of disease and predicts a guarded outcome. At the same time, based on such information, specific treatment, that is, chemotherapy, radiation, and surgery can be initiated.

1.2. GENERAL ASPECTS

Molecular pathology generally describes the aspect of pathology that is removed from the purely histologic aspect of diagnosis and uses information on the molecular level for diagnosis and prediction of outcome. Thus, the molecular aspect of pathology deals with identification of genes and the subsequent change in cellular architecture and expression of proteins in a given disease. Taken in such broad terms, molecular pathology is something pathologists have done for a long time, even before biochemical techniques were invented to analyze cellular DNA. Application of molecular pathology was used to imply the analysis of cellular structures at the electron microscope level or the analysis of proteins within the cell (Roizin, 1964). Staying with malignant tumors, identification of specific proteins within tumor cells can aid in the diagnosis of cellular origin, which may be important for both diagnostic and therapeutic purposes. The presence of tumor within the lung that expresses prostate-specific antigen (PSA) will undoubtedly define this tumor as a metastatic lesion and exclude a pulmonary primary tumor. These proteins may be visualized both by immunohistochemistry, that is, staining with immunohistochemical stains for PSA in case of prostate carcinoma, or by histochemical methods, that is, visualization of mucins with mucicarmin stain for other lesions. These examples use the expression of normal proteins in a tumor, which is, naturally, gene driven. Further assessment of tumors can identify expression of proteins that are not normally expressed in normal cells and, again, be of diagnostic use. The wild type of the p53 protein is a short-lived protein. The probability to have wild-type protein present in a given cell at a given time is quite low, and, thus, staining of tissues that contain wild-type p53 will result in a negative staining. Mutated p53, on the other hand, has a long half-life, will be present in tissues containing that protein, and will stain positive for p53, indicating its abnormal presence (Rom et al., 2000).

1.3. MOLECULAR PATHOLOGY, THE MOLECULAR WAY

The genetic code represents a specific code of four desoxynucleotides, which combine with complementary strands of DNA. When isolated from the nucleus, DNA usually breaks easily at random areas, resulting in DNA strands of various