Molecular Pathology

A Practical Guide for the Surgical Pathologist and Cytopathologist

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CAMBRIDGE UNIVERSITY PRESS

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UNIVERSITY PRESS

University Printing House, Cambridge CB2 8BS, United Kingdom

Cambridge University Press is part of the University of Cambridge.

It furthers the University's mission by disseminating knowledge in the pursuit of education, learning and research at the highest international levels of excellence.

www.cambridge.org
Information on this title: www.cambridge.org/9781107443464

First published 2015

Printed in the United Kingdom by Bell and Bain Ltd

A catalogue record for this publication is available from the British Library

Library of Congress Cataloguing in Publication data
Molecular pathology (2015)
Molecular pathology : a practical guide for the surgical pathologist and cytopathologist / edited by John M. S. Bartlett, Abeer Shaaban, Fernando Schmitt.
p. ; cm.
Includes bibliographical references and index.
ISBN 978-1-107-08298-4 (Hardback) – ISBN 978-1-107-44346-4 (Mixed Media) – ISBN 978-1-316-01470-7 (ebook)
I. Bartlett, John M. S., editor. II. Shaaban, Abeer, editor. III. Schmitt, Fernando, editor. IV. Title.
[DNLM: 1. Pathology, Molecular–methods. 2. Molecular Diagnostic Techniques–methods. QZ 50]
RB113
616.07–dc23 2015022902

ISBN 978-1-107-44346-4 Mixed Media
ISBN 978-1-107-08298-4 Hardback
ISBN 978-1-316-01470-7 Cambridge Books Online

Contents

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Preface

The practice of pathology has undergone significant evolution over the last few years. Molecular pathology has been instrumental in driving refined classifications of tumors and providing prognostic and predictive information based on the molecular phenotype of those tumors. As the wealth of targeted therapies in development is applied to the treatment of tumors, the demand for molecular diagnostic testing in pathology will inevitably increase.

Molecular pathology has therefore become an integral part of the daily diagnostic practice of pathologists. As defined by the Medical Research Council (MRC), molecular pathology is a discipline that seeks to describe and understand the origins and mechanisms of disease at the level of macromolecules (for example, DNA, RNA and protein) largely using patient samples. Pathologists are often asked to provide a large amount of diagnostic and prognostic information derived from molecular testing of tissue samples or cytological preparations. Moreover, through identifying the shared molecular characteristics in groups/strata of patients (stratified medicine), better diagnostic tools and effective therapeutic measures could be developed with significant health and economic benefits.

This book is aimed towards general and specialist practising and trainee pathologists to serve as an everyday manual for the practising pathologist. It contains the essential molecular pathology information in a format relevant to the reporting pathologist. It provides an overall view of current molecular pathology techniques followed by separate chapters detailing their clinical applications in various tissues covering both histopathology and cytopathology. We believe this book will aid pathologists in their daily practice of providing essential information for better diagnosis and management of patients.

Glossary

Accuracy: In terms of biomarker measurements, accuracy is defined as how close the measurement of any particular analyte approaches its true value (see ASCO-CAP guidelines on HER2 2007). Such a definition presupposes the existence of a "gold standard" for the measurement of any particular analyte, which can represent a significant challenge to the demonstration of analytical accuracy in biomarker studies.

Adjuvant therapy: In the context of cancer therapy, adjuvant therapy is treatment, usually chemotherapy or radiotherapy, given to patients where the primary cancer has been eradicated (by surgery), but where there remains a statistical probability that undetected or undetectable disease persists elsewhere. It represents a clinical area where treatment is based not on evidence of disease, but on risk of disease.

AFIP: Armed Forces Institute of Pathology – US government institution, founded in 1862, which provides diagnostic consultation, education and research in pathology.

Allele: One of two or more variant DNA sequences occurring at a particular gene locus. Typically one allele is more common, and other alleles ("variants") are rare. Allelic variation may impact on gene function.

Allele-specific oligonucleotide (ASO): ASO is a short piece of synthetic DNA complementary to the sequence of a variable target DNA. It acts as a probe for the presence of the target in a Southern blot assay or, more commonly, in the simpler Dot blot assay.

Amplification refractory mutation system (ARMS): ARMS is a simple method for detecting any mutation involving single base changes or small deletions. ARMS is based on the use of sequence-specific PCR primers that allow amplification of test DNA only when the target allele is contained within the sample. Following an ARMS reaction, the presence or absence of a PCR product is diagnostic for the presence or absence of the target allele.

Amplification: An increase in the number of copies of a gene or DNA fragment usually as a result of a chromosomal alteration during tumor pathogenesis.

Amsterdam Criteria (see also Bethesda guidelines): The Amsterdam Criteria were developed following a consensus which arose out of the 1990 meeting of the International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer (ICG-HPNCC) or Lynch syndrome in order to assist clinicians in the identification of individuals or families who were likely to suffer from this condition.

Aneusomy: This describes the condition where there exists greater than two copies of a whole chromosome or chromosomal region in comparison to the normally expected two copies per cell in the human genome.

Annealing temperature: The temperature that allows for the formation of hydrogen bonds between complementary bases in a sequence and hence allows for hybridization.

Annealing: The process wherein complementary bases (e.g. dATP↔dTTP and dCTP↔dGTP) within a nucleic acid sequence align following formation of hydrogen bonds to create a double-stranded molecule.

ASCO guidelines: Guidelines developed under the auspices of the American Society of Clinical Oncology.

ASCO-CAP guidelines: Guidelines, usually with regard to diagnostic procedures developed by groups of experts under the auspices of the American Society of Clinical Oncology and the College of American Pathologists.

Automated Childhood Cancer Information System (ACCIS): An authoritative source of European data on cancer incidence and survival of children and adolescents.

Autosomal dominant: A disease trait where an inherited allele from only one parent is sufficient to confer risk of disease or disease itself. In this case, the second parental allele is recessive.

Autosomal: Pertaining to any of the paired (i.e. non-sex) chromosomes within a cell.

Bacterial Artificial Chromosome (BAC): BAC describes the use of a construct where a fragment of the desired DNA (between 150 and 300 kb) can be inserted into the F-plasmid and transformed into *Escherichia coli* (*E.coli*). Transformed *E.coli* are cultured and the DNA extracted to be used for molecular analyses.

Base pair: Two DNA nucleotides paired together in double-stranded DNA. When used as a quantity (e.g. 8 base pairs, or 8 bp), this term refers to the length of a nucleotide sequence.

Bethesda guidelines (see also Amsterdam Criteria): The Bethesda guidelines were developed by the American National Cancer Institute (NCI) as a means of screening for individuals who should be counseled to undergo testing for HNPCC or Lynch syndrome related tumors.

Biliary intraepithelial neoplasia (BilIN): BilIN is a precursor lesion of hilar/perihilar and extrahepatic cholangiocarcinoma. BilIN represents the process of multistep cholangiocarcinogenesis and is the biliary counterpart of pancreatic intraepithelial neoplasia (PanIN).

Biochips, Chips or Microarray: This describes the use of a solid substrate, such as glass or polystyrene plastics, as a matrix for binding test materials, typically DNA or RNA probes, such that several (hundred or thousand) tests can be performed at one time in a high-throughput manner.

Bioinformatics: This is a discipline of computer science applying the use of computer programming to develop strategies for the analysis and management of high-level biological data.

Biomarker: Any substance, structure or process that can be measured in the body or its products which may influence or predict the presence, type or outcome of disease, the effect of treatments, interventions and even unintended environmental exposure, such as to chemicals or nutrients. NIH definition – "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention."

Blastema: An aggregate of histologically undifferentiated, typically "small round blue cells." Blastema can differentiate into different cell types/tissues.

Bouin fixative: This is a compound fixative used in histology, composed of picric acid, acetic acid and formaldehyde in an aqueous solution.

BRAF: BRAF is a member of the Raf kinase family of growth signal transduction protein kinases. The protein plays a role in regulating the MAP kinase/ERK's signaling pathway, which affects cell division, differentiation and secretion. Mutated BRAF, especially of the V600E BRAF, is seen in melanomas and also benign naevi. Drugs that inhibit mutated BRAF have been approved for treating late-stage melanoma.

Break-apart FISH probes: Dual colour DNA probes with a 3' and 5' component mapping either side of the breakpoint of specific genetic rearrangement, which appear as a fusion signal on a normal chromosome. Genomic rearrangement within tumor cells separates the probes, allowing visualization of the two component parts as distinct entities and distinctly separate colors.

Brightfield microscopy: Microscopy using transmitted white light.

CAP/IASLC/AMP lung biomarker guidelines: The College of American Pathologists (CAP), the International Association for the Study of Lung Cancer (IASLC) and the Association for Molecular Pathology (AMP) set out to create standardized international guidelines for lung cancer biomarker testing.

CAP: The College of American Pathologists.

Catalogue of somatic mutations in cancer (COSMIC): a website (http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/) designed to store and display somatic mutation information and related details of human cancers. The mutation data and associated information are extracted from the primary literature and entered into the COSMIC database. Data from The Cancer Genome Atlas and the International Cancer Genome Consortium portals are also uploaded into COSMIC.

CDK4 gene: Cyclin-dependent kinase 4 gene, also known as cell division protein kinase 4, is an enzyme that is encoded by the CDK4 gene. CDK4 is a member of the cyclin-dependent kinase family.

Cell-block: A paraffin block, suitable for sectioning, staining and microscopic study, prepared from any suspension of cells in fluid (i.e. aspirates or washings); cells are concentrated by centrifugation or filtering, and the resulting aggregation is processed as if it were a solid specimen of tissue.

Centromere: This is described as the part of the chromosome where sister chromatids are most closely attached, and visualized as a constricted region. Centromeres are composed of DNA with unique repetitive DNA sequences called satellite repeats. Each chromosome contains one centromere and these are often targets for enumeration assays.

Chimaeric fusion proteins: Novel oncogenic proteins, often tumor-specific, formed from the rearrangement and fusion of two genes, most usually by a chromosome translocation.

Chromatin immuoprecipitiation (ChIP): This describes a type of immunoprecipitation experimental technique used to investigate the interaction between proteins and DNA. The DNA-protein complex is precipitated using antibodies mobilized to a substrate. The protein is then unlinked from the DNA, and the subsequent DNA is extracted and analyzed.

Chromogenic *in situ* hybridization (CISH): This describes the detection of DNA or RNA in tissues or cells using specific probes that are visualized by bright-field microscopy.

Chromosomal rearrangement: This describes the disruption of the chromosome that results in the gain, loss or physical translocation/rearrangement of chromosomal material.The rearrangement may occur within the same chromosome (intrachromosomal), or between chromosomes (extrachromosomal, or chromosomal translocation). Rearrangements may be relatively simple or complex and involve several chromosomes.

Chromosomal translocation: This is a chromosome abnormality caused by rearrangement of varying-sized parts between non-homologous chromosomes. A gene fusion may be created when the translocation joins two otherwise separated genes: this is commonly observed in cancer, particularly NHL. Chromosomal translocations are detected on cytogenetics (e.g. by using fluorescence *in situ* hybridization, FISH) or a karyotype of the affected cells. Translocations can be **balanced** (in an even exchange of material with no genetic information extra or missing, and ideally full functionality) or **unbalanced** (where the exchange of chromosome material is unequal, resulting in extra or missing genes).

Chromosome instability (CIN): This is a phenotype where increased mis-segregation of chromosomes at mitosis leads to increasingly variable chromosome numbers within tumor cells. The phenotype is frequently a result of deregulation of key cell cycle checkpoints, especially those at G2/M. CIN is associated with increased sensitivity to DNA-damaging agents.

Glossary

Circulating tumor cells: As cancerous cells within a tumor proliferate, they do not all stay in a neighborhood. Some tumor cells shed into the vasculature from a primary tumor and circulate in the bloodstream. These circulating tumor cells (CTCs) can remain loose in circulation, cluster together as they travel, or lodge themselves in new tissues. CTCs thus constitute seeds for metastasis in distant organs, a mechanism that is responsible for the vast majority of cancer-related deaths. The common origin of CTCs means that they hold information about the primary tumor. Detection of CTCs in peripheral blood can serve as a "liquid biopsy" approach, which can be used for diagnosis and disease monitoring.

Class switch recombination: A biological mechanism that changes a B lymphocyte's production of immunoglobulin (i.e. antibodies) from one class to another, such as from the isotype IgM to the isotype IgG. During this process, the constant-region portion of the immunoglobulin heavy chain is changed, but the variable region of the heavy chain stays the same (the terms "variable" and "constant" refer to changes or lack thereof between antibodies that target different epitopes). Since the variable region does not change, class switching does not affect antigen specificity. Instead, the antibody retains affinity for the same antigens, but can interact with different and additional effector molecules.

Clone: An identical copy of a DNA sequence or entire gene; one or more cells derived from and identical to a single ancestor cell OR to isolate a gene or specific sequence of DNA.

Comparative Genomic Hybridization (CGH): This describes a method of comparing two differentially labeled DNA for the changes in copy-number between them. A typical experiment compares equal amounts of the test DNA (i.e. tumor) to a reference (i.e. normal) DNA by ascertaining the differences at a particular genomic location. This is normally achieved by the changes in the ratio of the emitted fluorescence by the test DNA vs. the reference DNA.

Copy-number Variation (CNV): CNV refers to the genetic trait involving the number of copies of a particular gene present in the genome of an individual. Genetic variants, including insertions, deletions and duplications of segments of DNA, are also collectively referred to as CNVs. CNVs account for a significant proportion of the genetic variation between individuals. Also called copy-number variant or copy-number aberration (CNA).

Core needle biopsy (CNB): CNB is a diagnostic procedure that uses a larger, hollow needle to withdraw small cylinders (or cores) of tissues, maintaining the architectural structure.

Coverage: The proportion of a target sequence (a single gene, a whole genome, etc.) sequenced. Coverage is sometimes used interchangeably with depth, when referring to "average coverage."

CpG island methylator phenotype (CIMP): CIMP is a phenomenon where multiple CpG islands are hypermethylated in cancer (first described in colorectal cancer), leading to the putative deregulation of key genes.

Cryoarrays: Tissue microarrays of snap-frozen tissues in OCT. They allow performing studies that are very difficult on FFPE tissues (e.g. proteomic studies, RNA hybridization studies).

DCIS: Ductal Carcinoma *in situ* of the breast.

Deletion: A genetic alteration that occurs when a segment of DNA is absent. The size of the segment can range from a single base to multiple genes.

Denaturation: The process of applying heat to break bonds between complementary bases, which unwinds DNA and leads to the separation of a double strand into two single-stranded molecules.

Depth: The average number of times base pairs in a region are sequenced. Depth can also refer to the number of times a single position was sequenced. Sometimes used interchangeably with coverage.

DFSP (dermatofibrosarcoma protuberans): An uncommon skin tumor arising in the deeper layer of the skin (the dermis). It grows slowly and has a tendency to recur after excision, but rarely metastasizes.

Diagnostic biomarker: This biomarker gives an indication if a disease exists or not.

DNA methylation: An epigenetic mechanism for negatively regulating gene expression through methylation of CpG islands within gene promoters; tumor cells often have hypermethylation of tumor suppressor genes and hypomethylation of oncogenes.

Double minute chromosome (DMs or dims): DMs are small fragments of extrachromosomal DNA that have the ability to replicate and are a means by which DNA may become amplified. These are seen as multiple extrachromosomal paired structures. Frequently, double minute chromosomes harbor oncogenes.

Down's syndrome: Human genetic syndrome associated with an additional copy of chromosome 21.

Driver gene: Any gene key to the development or progression of cancer.

Driver mutation: A mutation in a driver gene that results in a deleterious effect on the protein and confers the cell with a survival advantage.

EGFR tyrosine kinase inhibitors (TKIs): These drugs occupy the TK adenosine triphosphate (ATP) binding site, thereby preventing EGFR activation and downstream signaling effects.

Endobronchial ultrasound-guided fine-needle aspiration (EBUS-FNA): This is a safe and minimally invasive bronchoscopic technique that allows both visualization and cytologic sampling with a high diagnostic yield in a patient with mediastinal lymphadenopathy. Besides the most common indication of staging for a patient with a primary lung carcinoma, EBUS-FNA can be used to identify benign infectious and non-infectious processes, as well as lymphoma and malignancy of unknown primary.

Enzyme-linked Immunosorbant Assay (ELISA): ELISA describes the detection of a protein typically in a liquid sample such as urine, serum or blood, using the antibody-antigen interaction. Standard ELISA entails the mobilization of the capture antibody to a substrate such as a plastic multi-welled plate. The sample to be tested is

added to the well, allowing any antigens in the sample to be captured by the mobilized antibody. The captured antigen is then detected using an antibody conjugated to a detection system, such as a chromogen or fluorescent molecule.

Epidermal growth factor receptor (EGFR) mutation analysis: Part of the current standard of care in advanced non-small cell lung cancer (NSCLC).

Epigenetics: This describes the study of gene expression changes that are not due to modifications to the DNA sequence. Most commonly, epigenetics refers to the level of methylation experienced at cytosine residues.

Exon: The sequence of DNA present in mature messenger RNA, some of which encodes the amino acids of a protein. Most genes have multiple exons, with introns between them.

Extension: The addition of nucleic acid bases during nucleic acid replication.

Fallopian tube tumors: Neoplastic tissue originates from Fallopian tubes. Benign tumors (adenomatoid tumors) are originated from the subserosal area. Tubal adenocarcinomas are rare and are often associated to the BRCA germline mutation. Fallopian tube cells are also involved in ovarian carcinoma origin.

Familial adenomatous polyposis: An inherited condition in which numerous polyps (growths that protrude from mucous membranes) form on the inside walls of the colon and rectum. It increases the risk of colorectal cancer. Also called familial polyposis and FAP.

Familial colorectal cancer type X syndrome (FCC-XS): This term is often used to describe apparently familial or Lynch syndrome colorectal cancers where no underlying mismatch repair gene mutation or defect can be identified. To fall within this grouping, cancers must satisfy the "Amsterdam criteria," but have no evidence of mismatch repair defects.

Fine-needle aspiration (FNA): FNA biopsy (FNAB) or FNA cytology (FNAC) is a diagnostic procedure used to investigate superficial lumps or deep masses. In this technique, a thin needle is inserted into the lesion for sampling of cells that, after being stained, will be examined under a microscope.

Flow cytometry: This is a laser-based, biophysical technology employed in cell counting, cell sorting, biomarker detection and protein engineering, by suspending cells in a stream of fluid and passing them by an electronic detection apparatus. It allows simultaneous multiparametric analysis of the physical and chemical characteristics of up to thousands of particles per second. This technique is used in the diagnosis of several diseases, especially lymphoproliferative diseases, but has many other applications in basic research and clinical practice.

Fluorescence *in situ* hybridization (FISH): FISH describes the detection of DNA or RNA in tissues or cells using specific probes that are visualized by fluorescence microscopy. The genomics or cellular location of these labeled probes to their targets is achieved by complementary base pairing. It is important for visualizing and mapping of chromosomal abnormalities and rearrangements. FISH can also be used to determine how many chromosomes of a certain type are present in a cell. For FISH, small DNA strands called probes that have a fluorescent label attached and that are complementary to specific parts of a chromosome are used to look at specific areas of a chromosome. FISH does not need to be performed on cells that are actively dividing, which makes it a very versatile procedure that can be performed on formalin-fixed and paraffin-embedded tissues.

Fluorescence microscopy: Fluorescence microscopy uses fluorescence to generate the image. It makes use of filters to excite the fluorescent molecule at a particular wavelength, enabling it to emit a signal within the visual or non-visual range.

Food and Drug Administration (FDA): The US Food and Drug Administration is responsible for the regulatory oversight and licensing of new diagnostic tests and therapeutics (among other roles) in the United States.

Formalin-fixed paraffin-embedded (FFPE): A method of tissue preservation and storage wherein tissues are fixed using a formaldehyde-based medium (formalin) and embedded in a wax matrix (paraffin) that permits tissue sectioning.

Fusion probes: This describes the use of differentially labeled probes from two different chromosomal regions for the detection of a translocation event between them. When the two probes are co-localized, the fusion event has occurred. When the two probes are separate, the fusion event has not occurred.

Gardner syndrome: This is an autosomal dominant form of polyposis characterized by the presence of multiple polyps in the colon, together with tumors outside the colon. The extracolonic tumors may include osteomas of the skull, thyroid cancer, epidermoid cysts and fibromas, as well as the occurrence of desmoid tumors in approximately 15 percent of affected individuals.

Gastrointestinal stromal tumor (GIST): GIST is a neoplasm that arises in the smooth muscle pacemaker cells of Cajal. It is pathologically identified by a tyrosine kinase membrane receptor, c-kit protein (CD 177 antigen). Most occur in the stomach, and gastric GISTs have a lower malignant potential than tumors found elsewhere in the GI tract.

GCP: Good Clinical Practice is a system of quality management governing the performance of clinical research, including clinical trials, involving human subjects. The principles of GCP encompass ethical practices, documentation, privacy and all associated aspects of clinical research involving human subjects. Increasingly, where translational laboratory research is allied to clinical trials, this is linked to Good Laboratory Practice in a combined system of Good Clinical and Laboratory Practice.

Gene expression profiling: This is the measurement of the activity (the expression) of thousands of genes at once, to create a global picture of cellular function. These profiles can, for example, distinguish between cells that are actively dividing, or show how the cells react to a particular treatment. Many experiments of this sort

Glossary

measure an entire genome simultaneously, that is, every gene present in a particular cell.

Germline mutation: A gene change in a body's reproductive cell (egg or sperm) that becomes incorporated into the DNA of every cell in the body of the offspring. Germline mutations are passed on from parents to offspring and are present in every cell. They are also called hereditary mutation.

GLP: Good Laboratory Practice is a system of quality management and assessment of quality through quality controls implemented in research organizations and other bodies carrying out laboratory research. Implicit in this process is the assumption that: (a) quality management is an essential component of both pre-clinical and clinical research; and (b) all diagnostic procedures, which are regulated by other bodies in addition, will nonetheless adhere to GLP.

Hamartomatous polyposis syndromes: These are a heterogeneous group of disorders that share an autosomal-dominant pattern of inheritance and are characterized by hamartomatous polyps of the gastrointestinal tract. These syndromes include juvenile polyposis syndrome, Peutz-Jeghers syndrome and the PTEN hamartoma tumor syndrome. The frequency and location of the polyps vary considerably among syndromes, as does the affected patient's predisposition to the development of gastrointestinal and other malignancies.

Hereditary non-polyposis colorectal cancer (HNPCC): See also Lynch syndrome. HNPCC is an autosomal dominant genetic condition that has a high risk of colon cancer as well as other cancers, including endometrial cancer (second most common), ovary, stomach, small intestine, hepatobiliary tract, upper urinary tract, brain and skin. The increased risk for these cancers is due to inherited mutations that impair DNA mismatch repair.

High-resolution melting analysis (HRMA): HRMA is a post-PCR analysis method used to identify variations in nucleic acid sequences. The method is based on detecting small differences in PCR melting (dissociation) curves. It is enabled by improved dsDNA-binding dyes used in conjunction with real-time PCR instrumentation that has precise temperature ramp control and advanced data capture capabilities. Data are analyzed and manipulated using software designed specifically for HRM analysis.

Hodgkin's lymphoma (HL): HL was first described by Thomas Hodgkin in 1832 and later classified by Robert Luke in 1963. There are four particular subtypes of classic HL (excluding nodular lymphocyte predominant HL), based on the Reed-Sternberg cell morphology and accompanying reactive cell infiltrate and degree of sclerosis. HL tend to be more radiation sensitive than NHL.

Homebox genes: A large family of genes directly involved with human embryonic development. They can also regulate the activity of other genes, acting as transcription factors, and tumor suppressors. Deregulated activity or mutations of these genes are associated to the malignancies development.

HPV (Human papillomavirus): More than 150 types of this virus are currently identified which generally correlate with the three clinical categories applied to HPV infection: anogenital or mucosal, non-genital cutaneous, epidermodysplasia verruciformis. HPV is common: most people have the virus at some time in their lives. In most cases, the virus causes no symptoms and goes away on its own. Some types of HPV can cause changes in the cells of the cervix or the lining of the mouth and throat. They are known as high-risk HPVs. The changed cells have an increased risk of becoming malignant. Other types of HPV can cause warts, but do not usually cause cell changes that develop into cancer. They are therefore called low-risk HPVs.

HPV testing: Use of molecular identification of high-risk HPV for screening proposals in order to enhance the sensitivity of cervical lesions detection generally underestimated by conventional Pap test. The specificity is supposed to also be improved with cytology reflex test for cases tested positive for HPV.

ICGC: The International Cancer Genome Consortium is a collaborative network of cancer genome projects, including the TCGA (see TCGA). The goal of the ICGC (see ICGC.org) is "to obtain a comprehensive description of genomic, transcriptomic and epigenomic changes in 50 different tumor types and or subtypes which are of clinical and societal importance across the globe."

Immunohistochemistry (IHC): An immunostaining technique to detect protein expression in tissue sections by the use of antibodies that bind specifically to antigens in tissues and are visualized by a marker such as fluorescent dye, enzyme or colloidal gold. Immunohistochemistry is widely used in the diagnosis of malignant cells, to study the localization of biomarkers and differentially expressed proteins in different tissues.

***In situ* hybridization (ISH):** A method detecting mRNA or DNA *in situ* in tissue or cell block sections using chromogenic or fluorescent probes, allowing signal localization to specific cell populations.

Indels: Mutations that result in the insertion and/or deletion of nucleotides.

Interphase cells: This describes the state of nuclei within tissues that are not undergoing mitosis.

Intraductal papillary mucionous neoplasia (IPMN): Intraductal grossly visible (1 cm or more) epithelial neoplasm of mucin-producing cells, arising in main pancreatic duct or its branches; neoplastic epithelium is usually papillary; variable mucin secretion, duct dilatation (cyst formation) and dysplasia; classify based on the highest degree of cytoarchitectural atypia and invasiveness as IPMN with low- to intermediate-grade dysplasia, IPMN with high-grade dysplasia and IPMN with associated invasive carcinoma.

Intrinsic subtypes: molecular subtypes of breast cancer based on microarray gene expression patterns that improve prognostication and prediction of response to therapy compared with categories defined by classical clinicopathological characteristics.

Karyotyping: The classical method for identifying chromosome rearrangements in tumors. Almost all specific translocations known in soft tissue tumors have been detected using this method.

Ki-67: This is a cellular marker strictly associated with cell proliferation. During interphase, the Ki-67 antigen can be exclusively detected within the cell nucleus, whereas in mitosis most of the protein is relocated to the surface of the chromosomes. Ki-67 protein is present during all active phases of the cell cycle (G_1, S, G_2 and mitosis), but is absent from resting cells (G_0).

Kleinfelter syndrome: Human genetic syndrome affecting males where there is an extra chromosome X, in addition to the normal X and Y chromosome per cell.

Laser capture micro-dissection (LCM): The use of a laser to precisely dissect tissues under a microscope.

Liquid-based cytology: This is a method of preparing samples for examination in cytopathology. The sample is collected, by a small brush or needle, in the same way as for a conventional smear test, but rather than the smear being transferred directly to a microscope slide, the sample is deposited into a small bottle of preservative liquid.

Ligation: The enzymatic process of joining two DNA sequences by creating new phosphodiester bonds.

Loss of heterozygosity (LOH): This describes the condition where there is the loss of a copy of a given locus, gene or chromosomal region.

Lynch syndrome (see HNPCC)

Malignant fibrous histiocytoma (MFH): A type of sarcoma arising in bone and/or soft tissue. Controversy exists as to its cell of origin. In 2002, the World Health Organization (WHO) declassified MFH as a formal diagnostic entity and renamed it as an undifferentiated pleomorphic sarcoma not otherwise specified (NOS).

Massively parallel sequencing: Another term for next generation sequencing.

MDM2 gene: Murine double minutes 2, a gene that is commonly amplified in well-differentiated liposarcoma.

Melting temperature: The temperature at which 50 percent of the oligonucleotide primers are bound to their complementary sequence and the other 50 percent are separated into single-stranded molecules.

MEN (Multiple Endocrine Neoplasia): Familial syndromes that are attributable to germline mutations in tumor suppressor genes. These syndromes are inherited as autosomal dominant tumor syndromes with variable penetrance.

Metabolomics: A method by which the levels of different metabolites (sugars, lipids, phosphate-containing substances and others) are quantitatively measured; often altered in tumor cells compared to normal cells, as well as following tumor-targeting therapy, such as radiation and chemotherapy.

Metaphase spreads/cells: This describes the preparation of cells that enables the chromosomes to be visualized at metaphase. Mitotically active cells are inhibited at metaphase and fixed in solution to maintain chromosomal architecture.

Metastasis: This is the spread of a cancer disease from one organ or body part to another not directly connected with it.

Microarrays: This is a multiplex lab-on-a-chip. It is a 2D array on a solid substrate (usually a glass slide or silicon thin-film cell) that assays large amounts of biological material using high-throughput screening miniaturized, multiplexed and parallel processing and detection methods. There are many types of microarrays, including DNA microarrays, protein microarrays and tissue microarrays.

MicroRNA: A small non-coding RNA molecule (containing about 22 nucleotides) found in plants, animals and some viruses, which regulates gene expression by binding to the 3'-untranslated regions (3'-UTR) of specific mRNAs.

Microsatellite instability (MSI): MSI is one genotypic consequence of impaired DNA mismatch repair. It is detected by the presence of multiple errors in short repetitive DNA sequences (either mononucleotide or dinucleotide repeats or microsatellites).

Mismatch repair (MMR): MMR is a form of DNA repair which corrects errors in DNA replication or which arise as a result of faulty recombination or DNA damage. During normal replication, the daughter strand may contain errors and this form of DNA repair uses the parental strand as a template to correct mismatches in DNA sequence which result from such errors.

Missense mutation: A mutation that results in a different amino acid sequence.

Monoclonal cells: These are defined as a group of cells produced from a single ancestral cell by repeated cellular replication. Hence, they are suggested to form a single clone. Monoclonality testing using IgH-PCR or TCR-PCR is one way of providing additional evidence to morphology and immunohistological studies to define a lymphoproliferative lesion as being malignant (or not).

Mononucleotide repeat: These comprise multiple repeats of a single nucleotide in DNA and are frequently used in assays to diagnose mismatch repair syndromes. Due to the nature of long mononucleotide repeat sequences, they are prone to extension or deletion during DNA synthesis and a failure to detect and repair such errors is indicative of mismatch repair syndrome.

Monosomy: This describes the condition where there exists one copy of a whole chromosome or chromosomal region in comparison to the normally expected two copies per cell.

Mucinous cystic neoplasias (MCN): Benign or potentially low-grade malignant cystic epithelial neoplasm composed of cells which contain intracytoplasmic mucin (WHO). MCN is one of the three precursor lesions of pancreatic adenocarcinoma (see also PanIN, IPMN).

Multi-colored FISH (MFISH): MFISH describes the detection of more than two differentially fluorescently detected/labeled DNAs or RNAs in tissues or cells, by fluorescence microscopy.

MYH-associated polyposis: This is a condition predisposing to colorectal cancer, caused by germline mutations in the base excision repair (BER) gene

Glossary

MUTYH (MYH). The phenotype is often undistinguishable from that of autosomal dominant familial adenomatous polyposis (FAP). The number of adenomas is often lower and affected patients are often sporadic cases. Biallelic MUTYH mutations have also been detected in patients affected with early-onset colorectal cancer (CRC) without polyps. Cancers are more frequently located in the proximal side of the colon compared to APC-related FAP. Generally, mean age at diagnosis of MAP is 48 to 56 years, later than in APC-related FAP.

NATA: National Association of Testing Authorities (Australia) is one of a number of national bodies responsible for overseeing adherence by members (in this case diagnostic laboratories) to both national and international standards.

NET (neuroendocrine tumor): NETs are a heterogenous group of neoplasms arising from neuroendocrine cells of the diffuse endocrine system. They can be seen in a variety of anatomic locations and although their morphologic features and hormonal activities can be similar, their pathogenesis appears to vary with the site.

Next generation sequencing (NGS): Also known as high-throughput sequencing, NGS is the "catch-all" term used to describe a number of different modern sequencing technologies, which allow us to sequence DNA and RNA in a massively parallel fashion (vs. single or a few DNA fragments) and more cheaply than the previously used capillary electrophoresis Sanger sequencing. As such, NGS has revolutionized the study of genomics and molecular biology.

Non-Hodgkin's lymphoma (NHL): A group of over 80 diverse malignancies that arise from lymphocytes, a type of white blood cell, normally found within the blood and tissues. The NHLs vary in their morphology, immunophenotype, genetic alterations and in the degree of clinical severity. They are distinct from Hodgkin's lymphoma.

Nonsense mutation: A mutation that results in a stop codon, which may produce a truncated protein.

NSCLC subtyping: The subtyping of NSCLC into adenocarcinoma and squamous cell carcinoma is crucial and may require beyond microscopy the use of specific immunostainings.

Oligonucleotides: Described as short, single-stranded DNA or RNA molecules that are synthesized for various molecular applications.

Oncogene: A gene involved in promoting cell growth or proliferation, which if altered can promote cancer.

Ovarian cancer: Ovarian epithelial carcinomas count for the majority of ovarian cancer and are divided into serous, endometrioid, mucinous or clear-cell carcinoma types. Other less frequently seen ovarian cancers include germ cell tumor, dysgerminoma, yolk sac tumor, teratoma and sex cord-stromal tumor.

Pancreatic intraepithelial neoplasia (PanIn): PanIn are the most common precursor lesions of pancreatic ductal adenocarcinoma; they are microscopic papillary or flat, non-invasive epithelial neoplasms that are usually <5 mm and confined to pancreatic ducts; they are composed of columnar to cuboidal cells with variable mucin, and are divided into three grades according to the degree of cytological and architectural atypia.

Passenger mutation: A mutation that does not result in a deleterious effect and does not confer the cell with a survival advantage.

PBS (phosphate buffer saline): PBS is a water-based salt solution containing sodium phosphate, sodium chloride and, in some formulations, potassium chloride and potassium phosphate. The osmolarity and ion concentrations of the solutions match those of the human body (isotonic).

Philadelphia chromosome (Ph): The name of a specific chromosomal translocation associated with chronic myelogenous leukemia (CML) resulting in the translocation between the ABL gene (9q32) and the BCR gene (22q11).

PDFGRA: Alpha-type platelet-derived growth factor receptor is a protein that is encoded by the PDGFRA gene. The gene is mutated in a subset of gastro intestinal stromal tumors (GISTS).

Photolithography: This describes a micromanufacturing process that is directed by light.

Phred score: A quality score (denoted "Q") related to the probability of an observation being an error. Phred scores are commonly used to describe the qualities of base calling and sequence mapping.

Polymerase Chain Reaction (PCR): A procedure that produces millions of copies of a short segment of DNA through repeated cycles of: (1) denaturation; (2) annealing; and (3) elongation. PCR is a very common procedure in molecular genetic testing and may be used to generate a sufficient quantity of DNA to perform a test (e.g. allele-specific amplification, trinucleotide repeat quantification).

Polymerase: An enzyme class that allows for the creation of DNA or RNA polynucleotides.

Predictive assay/biomarker: This identifies subpopulations of patients who are most likely to respond to a given therapy.

Predictive biomarker: Measurable indicator of a biological state which identifies patients who will benefit from a specific treatment.

Primer: A defined segment of DNA or RNA used to initiate replication of a sequence by DNA polymerase.

Probe: This refers to a known fragment of DNA or RNA (i.e. specific gene, locus) that is labeled and detectable by fluorescent or chromogenic means.

Prognosis (see also residual risk): The prognosis of a patient following a diagnosis of cancer is widely understood, correctly, to be a forecast of the likely course of the disease and its outcome. However, in its strictest context, prognosis relates to outcome *in the absence of treatment or intervention*. Almost no cancer patient is simply observed, all receive either treatment (be it surgery or chemo-/radiotherapy) or surveillance (for early prostate cancer). Frequently, therefore, when discussing *prognosis*, clinicians are in fact discussing "residual risk,"

i.e. the risk remaining after treatment of an adverse outcome. This is particularly important in an era of "residual risk assessment" using nonograms or diagnostic assays which may only apply in the context of the management of patients at the time they were developed.

Prognostic assay/biomarker: This indicates the likely course of the disease in an untreated individual.

Prognostic biomarker: Characteristics that can estimate the chance of survival or disease recurrence in the context of either no treatment or a previously performed treatment.

Promotor hypermethylation: Hypermethylation of cytosine residues within CpG islands located within or in proximity to promoters of regulating gene expression is one mechanism which is now understood to be important in the development of cancers. Hypermethylation of promoter regions of tumor suppressor genes can lead to "gene silencing" or loss of gene expression which may result, phenotypically, in a loss of gene expression similar to that seen following genetic loss through deletion.

Pyrosequencing: This is a method of DNA sequencing based on the "sequencing by synthesis" principle. It differs from Sanger sequencing, in that it relies on the detection of pyrophosphate release on nucleotide incorporation, rather than chain termination with dideoxynucleotides.

Real-time PCR: A PCR strategy that allows for the quantification of products as they are produced.

Receptor tyrosine kinases (RTKs): Surface molecules which induce cell survival and proliferation; often altered (mutated or amplified) in cancer, thereby extensively investigated for relevance in targeted therapy (e.g. EGFR, HER2, KIT).

Recombinant DNA: This describes the manipulation of DNA using the laboratory methods.

Reflex testing: A testing policy that does not require a separate clinician order for biomarker testing of lung tumors at diagnosis from patients presenting with stage I, II or III disease.

Residual risk (see also prognosis): Frequently confused with prognosis, residual risk is the risk of patients experiencing an adverse event (e.g. recurrence or death) after conventional treatments have been completed. While prognosis is an estimate of projected outcomes in the absence of treatments, "residual risk" implies the presence of a program of active interventions in the disease process including, but not limited to, surgery, radiotherapy and chemotherapy. Most "prognostic" algorithms or nonograms are in fact estimates of residual risk following conventional therapy.

Residual risk assay or nonogram: For many cancers, nonograms or residual risk assays (molecular diagnostic assays) may be of value in determining the risk/benefit of treatment interventions for certain groups of patients. Particularly in breast cancer, where multiple treatment options exist, such approaches are widely used to inform patients of the potential risks/benefits of different treatment options. Increasingly, molecular diagnostic approaches are being developed which claim to support such decisions.

Restriction fragment length polymorphism (RFLP): RFLP is a technique that exploits variations in homologous DNA sequences. It refers to a difference between samples of homologous DNA molecules that come from differing locations of restriction enzyme sites, and to a related laboratory technique by which these segments can be illustrated. In RFLP analysis, the DNA sample is broken into pieces (digested) by restriction enzymes and the resulting restriction fragments are separated according to their lengths by gel electrophoresis.

Ring chromosome: This describes a specialized chromosomal structure where the chromosome is in the shape of a ring and frequently contains several copies of a given DNA fragment.

ROSE: To enhance the adequacy rate of cytological samples for microscopic and molecular analysis, rapid on-site evaluation (ROSE) is performed at the time of sampling by a dedicated cytopathologist.

RPMI: This is a culture media containing a bicarbonate buffering system with variable amounts of amino acids and vitamins. It was developed at Roswell Park Memorial Institute, hence the acronym RPMI.

Sanger sequencing: The common name for capillary electrophoresis-based sequencing. Also called chain-termination sequencing, or first generation sequencing.

Segmental chromosome aberrations (SCA): Large-scale genomic dosage changes, often with loss or gain of whole chromosome arms.

Sensitivity (of a biomarker): The sensitivity of a biomarker test is a measure of how well the assay performs in picking up a disease or event (response to therapy, relapse, etc.) when the test is positive. Sensitivity can be calculated by dividing the number of true positives (test is positive and event occurs) by the sum of true positives and false negatives (where the test is negative but the event occurs).

Serous effusions: Accumulations of fluid within the peritoneal, pleural or pericardial space; associated with malignancy, as well as infectious, inflammatory and various other conditions.

Silent mutation: A mutation that results in no change in the translated amino acid sequence.

Silver *in situ* hybridization (SISH): SISH describes the detection of DNA or RNA in tissues or cells using silver particles that are visualized by bright-field microscopy.

Single Nucleotide Polymorphism (SNP): DNA variations that occur in over 1 percent of the population and do not cause disease. Each SNP represents a difference in one of the nucleotides adenine (A), thymine (T), cytosine (C) or guanine (G) in the genome and can differ between members of a species or paired chromosomes in an individual. Most SNPs have no effect on health or development. However, some SNPs are associated with certain diseases. These associations allow the evaluation of an individual's genetic predisposition to develop a disease.

Somatic hypermutation (SHM): SHM is a critical process used by B-cells to generate antibodies. The human immune system is highly adapted to generate an enormous diversity of antibodies against foreign

Glossary

pathogens using a limited set of genes within the genome. SHM has been highly conserved through mammalian biology and is the key process responsible for generating antibody diversity within B-cells.

Somatic mutation: A DNA alteration that occurs after conception. Somatic mutations can occur in any of the cells of the body except the germ cells (sperm and egg) and therefore are not passed on to children. These alterations can, but do not always, cause cancer or other diseases.

Specificity (of a biomarker): The specificity of a biomarker test is a measure of how well the assay performs in giving a true result for the population lacking disease or event (response to therapy, relapse, etc.) when the test is negative. Specificity can be calculated by dividing the number of true negatives (test is negative and event is absent) by the sum of true negatives and false positives (test is positive in the absence of event).

Substitution mutation: Also referred to as a point mutation, this is a genetic alteration where one nucleotide is exchanged for a different nucleotide. Depending on the effect on the translated protein, a substitution may be categorized as a silent, missense or nonsense mutation.

Targeted therapy: Type of treatment that acts by interfering with essential biochemical pathways or mutant proteins or tissue environment that contributes to cancer growth and survival.

Telomere: This describes the ends of sister chromatids where a specific DNA sequence exists to ensure protection of the chromosome ends during replication.

The Cancer Genome Atlas (TCGA, see also ICGC): A coordinated effort (http://cancergenome.nih.gov/) to accelerate our understanding of the molecular basis of cancer through the application of genome-wide analysis technologies, including large-scale genome sequencing. The TCGA Research Network has catalogued aberrations in the DNA (mutations, SNPs and methylation), mRNA and microRNA of thousands of tumors relative to matched normal genomes.

Thymidylate synthase (TS): TS is a key enzyme in the synthesis of 2'-deoxythymidine-5'-monophosphate, an essential precursor for DNA biosynthesis. For this reason, this enzyme is a critical target in cancer chemotherapy. As the first TS inhibitor in clinical use, 5-fluorouracil (5-FU) remains widely used for the treatment of several types of cancers.

Tissue microarray (TMA): Tissue microarrays are an ingenious means of collecting large numbers of samples from tumor specimens into a miniaturized tumor biobank. Usually, small punch core biopsies are taken from donor tissue blocks and assembled into a new recipient pathology block in an ordered and precise pattern. In this way, many hundreds or indeed thousands of tumor samples may be represented on a single tissue block to facilitate research. Increasingly, mini TMAs are used to assemble quality controls for conventional diagnostic approaches into a single strip which can, in some instances, be placed adjacent to the test sample, allowing "in slide" controls for comparison of staining or ISH.

TMPRSS2-ERG gene fusions: TMPRSS2-ERG is a fusion of pieces of DNA from two different genes, TMPRSS2 and ERG. TMPRSS2-ERG gene fusion causes the ERG gene that contributes to cancer cell growth, survival and movement to become abnormally activated by hormones (e.g. testosterone) in the prostate.

TMPRSS2-ETS gene fusions: Fusions between the promoter of the androgen-regulated transmembrane protease serine 2 gene (TMPRSS2) and erythroblastosis virus E 26 (ETS) transcription factors in prostate cancer. These gene fusions result in androgen-dependent transcription of ETS factors in prostate cancer cells. The most common fusion is with ERG (ETS-related gene), a member of the ETS family, resulting in the TMPRSS2-ERG gene fusion in approximately 50 percent of prostate cancer cases. TMPRSS2 has also been identified in fusions with the ETS family members ETV1, ETV4 and ETV5 in prostate cancer.

TNM staging: The TNM staging system is a consensus staging approach to multiple cancers coordinated and maintained by the UICC (Union for International Cancer Control) and also adopted by the AJCC (American Joint Committee on Cancer). TNM staging includes: (1) a measure of primary tumor size, including notation of spread beyond the site of origin; (2) measurement of spread to local lymph nodes; and (3) measurement of distant metastases which combined make up the tumor nodes metastasis or TNM classification of tumors.

Touton giant cell: A type of multinucleated histiocytic giant cell with nuclei arranged in a ring-like fashion that surround central homogenous eosinophilic or amphophilic cytoplasm.

Translocation: A chromosomal abnormality in which a chromosome breaks and a portion of it reattaches to a different chromosomal location.

Tumor cell enrichment: To avoid mutant allele dilution into wild-type DNA, cytological and histological samples undergo microdissection to select for the testing the most pure neoplastic cell population.

Tumor suppressor gene: A gene that normally suppresses cell growth, which if inactivated or deleted can promote cancer.

Tumor cellularity: The proportion of a tumor made up of tumor cells.

Tumor infiltrating lymphocytes (TILs): Lymphocytes which infiltrate into the tumor area are increasingly thought to reflect differential aspects of the "host response" to tumors. This aspect of tumor biology is attracting increasing research and clinical interest as the presence of TILs of different subtypes (e.g. T-helper or T-suppressor cells) is linked to treatment response and disease outcome.

Turcot syndrome: This is a genetic disease characterized by polyps in the colon (large intestine) in addition to tumors in the brain. Skin abnormalities can also occur. Turcot syndrome is inherited in an autosomal recessive manner and can result from mutations in either the adenomatous

polyposis coli (APC) gene or the mismatch repair genes underlying the syndrome of hereditary nonpolyposis colon cancer (HNPCC).

Turner syndrome: Also known as gonadal dysgenesis, this human genetic disorder affects females and is associated with the absence of one of two copies of the X chromosome per cell.

Type I endometrial carcinoma: Endometrial carcinoma (endometrioid and mucinous adenocarcinoma) that arise from the hyperplastic endometrium tissue are associated to estrogen activity, and show a favorable clinical outcome.

Type II endometrial carcinoma: Endometrial carcinomas (serous and clear cell carcinoma) arise from atrophic endometrium tissue, without estrogen association, and show an unfavorable clinical outcome.

Urothelial carcinoma: Urothelial carcinoma, also known as transitional cell carcinoma, is a malignant neoplasm derived from transitional epithelium, occurring mainly in the urinary bladder, ureters and kidney (renal pelvis). The cells lining these organs are called "transitional" because they can stretch and change shape without breaking. Urothelial carcinoma is the most common type of bladder cancer. Outside of the bladder, this is an uncommon cancer. It accounts for approximately 7 percent of kidney cancers. Urothelial carcinoma is the most common tumor of the renal pelvis.

Vogelstein model: A linear model of progression of colorectal cancer first proposed by Bert Vogelstein to explain the sequential development of mutations during the course of development and progression of colorectal cancer. Such linear models (applied to many cancers) are frequently referred to as "Vogelstein models." Like many models, this linear approach has been developed and expanded, including developments by Vogelstein himself, to include branched and other more complete model systems.

Whole-exome sequencing: A laboratory process that is used to determine the nucleotide sequence primarily of the exonic (or protein-coding) regions of an individual's genome and related sequences, representing approximately 1 percent of the complete DNA sequence.

Whole-genome sequencing: A laboratory process that is used to determine the sequence of nuclear DNA without enrichment for regions of interest. The human genome consists of approximately 3 billion nucleotides.

World Health Organization (WHO): An agency of the United Nations concerned with international health priorities across a broad spectrum of diseases, including cancer.

Xenograft: Tissue or organs from an individual of one species transplanted into or grafted onto an organism of another species, genus or family.