Technological advances in molecular pathology : a journey into the archives

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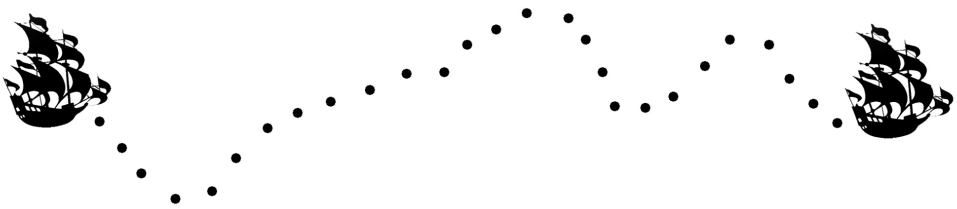
Chapter 8

Summary and “Nederlandse samenvatting”



Summary
This thesis describes technical advances in molecular tumor pathology that are related to the improved use of archives of formalin-fixed and paraffin-embedded tissue for molecular tumor diagnostics and research. The importance of isolating sufficient amounts of quality DNA from small fractions of FFPE material was confirmed, and we present examples of laboratory methodologies and analytical tools that have a large impact on cancer diagnosis, prognosis and therapeutics.

In **chapter 1**, important developments in pathology are described in a general and locally applied historical perspective. An overview of different pathological consultations is presented with a short introduction of the most commonly used molecular technologies in research and diagnostics. Analysis strategies, beginning with the arrival of the tissue in the laboratory to the reporting of the test results, are discussed using a DNA analysis workflow.

In **chapter 2**, we show that due to early diagnosis and adjuvant therapies, molecular tumor diagnostics has focused on the use of very limited pre-operative material. This makes high-quality DNA isolation challenging, particularly if the number of patients and the number of consultations per sample are increasing. A molecular analysis can only be performed if DNA of sufficient quality and quantity can be extracted from the archived tissue specimen. Therefore, we tested a fully automated DNA isolation system and compare it to our classical DNA isolation method. We determined that the fully automated method delivers high-quality DNA from small tissue cores and micro-dissected tissue. When the DNA is used in hydrolysis probe assays, we achieve a 24-hour faster turnover time, with 80% less hands-on time.

Multiplex ligation-dependent probe amplification (MLPA) is introduced in **chapter 3**. MLPA can be used to detect multiple chromosomal aberrations in a single experiment. We developed an MLPA-based assay to determine losses in FFPE tissue from oligodendroglial tumors (OG) and validated the MLPA results by comparing them with fluorescence in situ hybridization (FISH). The MLPA results were reproducible in all samples for which repeated experiments were performed, and we conclude that MLPA is a valid and reproducible method for the detection of 1p/19q chromosomal deletions in OGs.

For a reliable workflow in MLPA, tools are needed for administrative support, data management, normalization, visualization, reporting and interpretation. In **chapter 4**, we describe a MLPA data management system that was developed in-house in which a statistical approach is applied for the normalization and analysis of a large series of MLPA traces, making use of multiple control samples and internal controls. This integrated approach aids in the automated handling of a large series of MLPA data and guarantees a quick and streamlined dataflow from the beginning of the experiment to the authorized report.

The accurate detection of *KRAS* mutations is critical for the molecular diagnosis of cancer and may guide proper treatment selection. In **chapter 5**, we introduce a protocol for screening somatic mutations in *KRAS* that combines a whole-genome amplification (WGA) and a high-resolution melting analysis (HRM) as a prescreening method for samples harboring mutations. Direct Sanger sequencing is subsequently applied to define the specific *KRAS* mutations in the samples. We illustrate that this method is feasible for the screening of clinical specimens by analyzing pancreatic cancers and that it can be applied to virtually any potentially mutated region in the genome.

Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) and trans-esophageal ultrasound scanning with fine needle aspiration (EUS-FNA) are important techniques for the diagnosis and staging of NSCLC. In **chapter 6**, we demonstrate that allele-specific quantitative real-time PCR with hydrolysis probes can be accurately applied to EBUS and EUS fine needle cytological aspirates from NSCLC patients and that the mutations detected in the histological material of primary tumors can also be identified in cytological samples from the same patient.

Chapter 7 presents concluding remarks and provides future directions in molecular pathology. Major improvements in molecular technologies have been achieved in recent decades. The clinical value of different tests has been proven. Next-generation sequencing and digital pathology are challenging new technologies that will be implemented in due time in the clinic to achieve better and extended use of the current FFPE tissue archives and to the benefit of the individual patient.