

An introduction to Cancer Biomarkers in NSCLC – methods and platforms, clinical data and future aspects

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2.6 Real-time Polymerase Chain Reaction (PCR)

A low-throughput approach for measuring mRNA abundance is real-time polymerase chain reaction (qPCR) or its variants such as RT-qPCR or quantitative PCR (qPCR). With the method the expression of few genes are measured.

mRNA transcript signatures using both array technologies as well as PCR based techniques are already acting as "biomarker tools" in e.g. breast cancer and now already emerging in lung cancer.

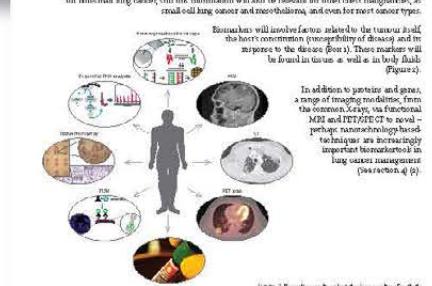
2.7 MicroRNA Analyses

MicroRNAs are single stranded RNA molecules that play a major role in gene regulation by binding to the 3' untranslated region (UTR) of target mRNAs. They are approximately 20-25 nucleotides long and have been developed for cancer research area of DNA. Preliminary

We will in this chapter outline some of the potential markers that we believe will be of importance in future care and clinical studies, both as outcome measure and treatment decision. We will focus on non-small lung cancer, but this information will also be relevant for other other malignancies, at least early stage cancer and most likely, also for most other cancer types.

Researchers will involve factors related to the tumor as well as the host (patient), and factors related to the disease (treatment). These markers will be found in tissue as well as in body fluids (Figure).

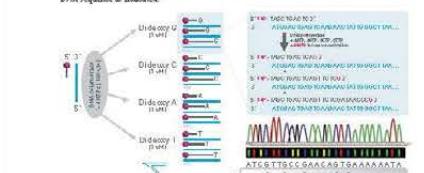
In addition to proteins and genes, a range of imaging modalities, functional assays, the latter key, molecular MRI and PET, seem to be needed – perhaps nanotechnology based techniques are increasingly important for early detection and lung cancer management (see section 4.9).



2. Technologies used in genomics

2.1 DNA Sequencing

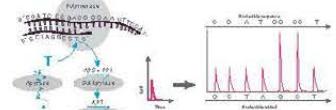
DNA sequencing is the process of determining the nucleic acid sequence of a given DNA fragment. This most often (DNA sequencing has been performed using the chain termination method developed by Sanger (page 5)). DNA fragments of different lengths as a result of chain termination due to incorporation of a deoxyribonucleotide (dNTP; dATP, dGTP, dCTP or dTTP) in four steps sequencing reaction. The length of the DNA fragments corresponds to the position of the different bases among the four bases (A, T, G, C). The base sequence of the different bands among the four lanes are then used to read from bottom to top the DNA sequence as indicated.



Chapter 1

Discovery of novel biomarkers using genomic and proteomic – molecular tools

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2.2 Comparative Genomic Hybridization Analysis

In epithelial human tumors, cells with large variations of the nucleotide sequence between chromosome aberrations and clones. Many solid tumors have a low mutational index, limiting the number of mutations needed for detailed chromosome banding analysis. The karyotype of epithelial tumors is usually not as stable as that of leukemic cells. Although often of allele loss, mutations, or DNA amplification, such methods might give only specific gains or losses in certain regions at a time, leaving other changes undetected. CGH can be used to detect changes in the genome that are not apparent to comparative genomic hybridization (CGH) analysis, and evaluates allele loss, gains, and DNA amplification in gene copy number alterations in a single experiment.

Optimal in situ hybridization of differentially labeled tumor DNA and normal homologous metaphase chromosomes (Figure 3). In *in-situ*-CAT a grid approach is used to hybridize tumor DNA and normal homologous chromosomes counter-labeled with chromosome at the hybridization step of the chromosomes between the tumor DNA and the normal tissue different statistical methods are given to determine about the presence of a change. The same approach is used in *in-situ*-CGH, so that to confirm as well as fresh specimen can be examined 100 µg of genomic DNA is needed for a genome wide analysis. To any type of tumor material (e.g. fine needle aspirate, paraffin tissue sections, tissue microarray) with high resolution, *in-situ*-CGH can identify subtle alterations of copy number imbalances. Whenever used to make informed decisions for the malignant cell population, and not DNA from normal cells, with abundance of DNA with 100% to achieve the *in-situ* range.

Figure 3: CGH - methodic applications, clinical data and future aspects

Chapter 3

it binds to RNA induced silencing complex (RISC) protein. The miRNA-RISC complex can bind to complementary sequences of mRNA and inhibit mRNA to be translated to proteins.

Several recent reports have provided evidence that miRNAs may act as key regulators of processes as diverse as early development, cell proliferation and cell death, apoptosis and metabolism, and carcinogenesis.

The miRNA array technology is basically the same as gene expression array methodology. A summary table of methods used in genetics can be seen in Table 1.

Table 1: Summary of methods used in genetics

Approach	Principle	Advantages	Disadvantages	Strength	Limitations
DNA Sequencing	Sequence each base one by one.	Can be used to detect point mutations, small insertions and deletions.	Expensive, time consuming.	Highly accurate.	Only suitable for short DNA sequences.
CGH	Comparative hybridization.	Can be used to detect gross chromosomal rearrangements.	Expensive.	Highly accurate.	Only suitable for short DNA sequences.
FOISH	Fluorescence in situ hybridization.	Identifies the presence of specific DNA sequences.	Expensive.	Only suitable for short DNA sequences.	Only suitable for short DNA sequences.

The use of biomarkers in the detection and treatment of lung cancer

1.1 Background
Cancer is a genetic disease at the molecular level leading to several cancers and a progressive alteration of cellular behavior. In addition, it is increasingly accepted that the host (patient) genotype and the tumor's surroundings influence the course of a cancer disease and the response of treatment (both radiotherapy and chemotherapy).

Tumor characteristics	DNA copy number DNA mutation screening Gene expression/RT-PCR profiling Genomic DNA methylation Circular RNA analysis Proteomic imaging
Patient characteristics	DNA sequencing (SNP) Gene expression/RT-PCR profiling Serum/plasma investigation Pharmacogenetics

During the past years, cancer has been extensively characterized and we know more than ever about cancer at the molecular level. This has led to the development of targeted therapies targeting specific molecules in a tumor ("targeted therapy"). In addition, we have experienced an intensive technological development, potentially enabling us to apply our knowledge about molecular processes in clinical practice.

However, there has been progress in the validation of biomarkers and the complex overall interaction between the tumor and the host. Such validation is essential before clinical application. The translation of biomarkers in clinical trials is therefore a prerequisite before implementation in clinical practice.

1.2 Clinical studies
Usually all steps in a clinical trial will potentially include evaluation of biomarkers (Figure 3). This should also be taken into account in the design phase, a careful selection of relevant biomarkers to be assessed throughout the study will lead to a better-structured research plan with power to give additional information about the patient. Also adverse events could be better understood if these biomarkers are taken seriously from the early planning phase.



We find it reasonable to divide the potential markers in two groups depending on their relation to either the tumor per se or to the patient as a whole. Classically, bolt-type of markers may be evaluated at each of the steps mentioned above.

Additionally, imaging as a biomarker is discussed in a separate paragraph, but this modality should undoubtedly also be employed at all trial steps.