Molecular-biological Diagnostics in Oncology

Malignant neoplasms are characterized by autonomous invasive growth of tumor cells and their ability to form distant metastases. Transformation of a normal cell into a tumor cell usually depends on the presence of a number of mutations in genes of different classes. Ionizing radiation, chemicals or biological agents (oncogenic viruses) are carcinogenic. **Inherited mutations** in specific genes (eg. tumor suppressor genes) can also cause the development of malignant cancer.

Sporadic and Familial Occurrence of Cancer

Sporadic occurrence, which is not related to genetic predisposition but to the action of external factors, significantly predominates in most tumors. Hereditary tumor syndromes, the development of which is mainly due to hereditary mutations in tumor suppressor genes, include, for example:

- Familial adenomatous polyposis (FAP) with hereditary ***APC*** gene mutations;
- Lynch syndrome (***h****ereditary* ***n****on****p****olyposis* ***c****olorectal* ***c****ancer* - **HNPCC**) with germline mutations in genes ***MSH2***, ***MLH1***, ***MSH6***, ***PMS1*** (*mismatch repair genes*);
- hereditary breast and ovarian cancer syndrome with ***BRCA1*** and ***BRCA2*** gene mutations;
- hereditary retinoblastoma with ***RB*** gene mutations;
- Li-Fraumeni syndrome with ***p53*** gene mutations.[1]

Hereditary tumors often develop at a younger age than sporadic tumors, are often bilateral, and tumor duplications are also typical. A higher number of genes may be responsible for the development of hereditary cancer. For example, predisposition to non-polyposis colorectal cancer occurs in inherited mutations in a number of genes that interfere with DNA repair processes.

You can find more detailed information on the Hereditary cancer syndromes page.

Tissue specificity varies significantly between mutations in individual tumor suppressor genes. For example, germline mutations in the ***RB*** gene are particularly associated with a risk (> 90%) of developing retinoblastoma (often bilateral in early childhood); in contrast, families with hereditary mutations of the ***p53*** gene have various tumors: breast tumors account for 28.1% of all malignancies, brain tumors 15.1%, soft tissue sarcomas 12.8%, osteosarcomas 12.3%, adrenal tumors 7.1%, hematological malignancies 3.2%, other tumors 21.4%[2].

In patients with a familial cancer, genetic testing confirms its hereditary origin. It then provides asymptomatic family members with information about the tumor predisposition (the risk of developing cancer). In people at high risk of cancer (mutation carriers), a **preventive screening program** can be designed for its early diagnosis.

Molecular-biological Diagnostics

Analyzed Material

The genetic material analyzed is usually genomic DNA; sometimes RNA is also tested. Genetic material is most often obtained from peripheral non-coagulated blood leukocytes or tumor tissue (obtained immediately after surgery or archived in paraffin blocks). DNA obtained from both tumor and normal tissue is analyzed to demonstrate *microsatellite instability* (MSI) or *loss of heterozygosity* (LOH) in tumor cells.

Types of Mutations

You can find more detailed information on the Mutations page.

Mutations can be defined as stable changes in the nucleotide sequence or arrangement of DNA. When germ cells are affected, they are passed on to offspring and can cause hereditary tumors. Somatic mutations are then important in the genesis of sporadic tumors. Depending on the type, mutations can be divided into **substitutions**, which represent transitions (A ↔ G or C ↔ T) or transversions (C/T ↔ A/G), **deletions**, **insertions**, **duplications**, and **extensive genomic deletions** and **rearrangements**. Extensive deletions or chromosomal translocations can lead to microscopically detectable structural changes in chromosomes. Mutations can also be divided according to the effect:

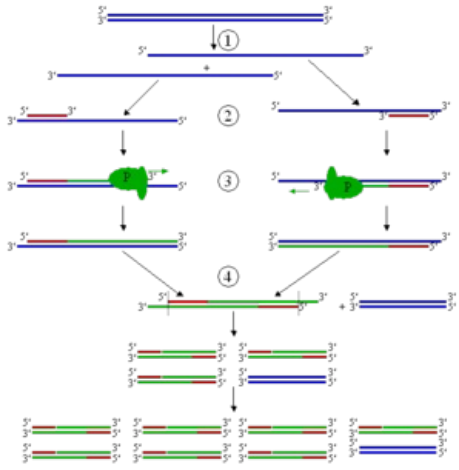
- **Synonymous mutations** do not change codon meaning and do not cause amino acid substitutions.
- **Missense mutations** change the sense of the codon, resulting in the incorporation of a different amino acid.
- **Nonsense mutations** result in a stop codon and therefore terminate translation process.
- **Shift mutations** lead to a frameshift and usually to a premature termination of protein synthesis.

Mutation Analysis

In the analysis of mutations, suitable gene fragments are usually first amplified by PCR (or RT-PCR) and their sequence analysis is then used to determine the mutation. When using prescreening assays in mutation analysis, subsequent sequencing serves to confirm and characterize the mutation. A number of methods can be used to prescreen mutations in genes responsible for tumorigenesis. **Restriction fragment length polymorphism**

analysis (RFLP-analysis) can be used to detect known point mutations (interfering with restriction enzyme recognition sequences), which typically occur, for example, in ras oncogenes. Electrophoretic methods, such as DGGE (**d**enaturing **g**radient **g**el **e**lectrophoresis) and SSCP (**s**ingle **s**trand **c**onformation **p**olymorphism), are standard techniques that detect changes in the nucleotide sequence of an amplified DNA fragment. In addition to pathogenic mutations, these methods also detect non-pathogenic polymorphisms or sequence variants whose significance may be unclear (some missense mutations).

Using **PTT** (*protein truncation test*) it is possible to detect pathogenic mutations leading to premature translation termination (nonsense mutations, shift mutations). Fragments amplified by PCR (RT-PCR) carrying the promoter phage sequence and the sequence necessary to initiate eukaryotic translation (*Kozak sequence*) serve in this technique as a template for in vitro RNA synthesis and subsequent translation (performed in the presence of a labeled amino acid). The synthesized proteins are further separated by polyacrylamide electrophoresis in the presence of SDS (SDS PAGE) and detected autoradiographically. Depending on the size of the translation products, it is possible to approximate the mutation. The method allows the analysis of long gene fragments (1000–2000 bp) and is used in the analysis of genes that are inactivated in most cases by mutations leading to protein truncation (eg *APC* gene, *BRCA1/BRCA2* genes).



PCR - Schematic drawing

MLPA and aCGH analysis

Extensive genomic deletions and rearrangements, which may account for more than 10% of pathogenic mutations in some tumor suppressor genes, are usually detected by *multiple ligation-dependent probe amplification* (**MLPA**)[3], eventually by *oligonucleotide array-based comparative genomic hybridization* (**aCGH**) analysis[4]. After delimiting the deletion, the truncated gene fragment carrying the deletion is amplified by PCR and the deletion (genomic rearrangement) is characterized by sequence analysis.

- In **MLPA analysis**, changes in gene copy number are detected based on differences in the hybridization of gene-specific oligonucleotide probes. Upon completion of hybridization, the exon-specific probes are ligated with DNA ligase, and after subsequent amplification, PCR products of different lengths are separated in a genetic analyzer. By comparing the sizes of the individual peaks, it is possible to quantify all exons of the respective gene and determine the extent of gene alteration.
- In **aCGH**, the gene copy number is determined based on the difference in hybridization between the analyzed and reference DNA. Using chromosome-specific aCGH with a high density of oligonucleotide probes, it is possible to locate gene alteration (deletion or duplication) relatively accurately.

Loss of Heterozygosity

Demonstration of the loss of heterozygosity of intragenic or nearby microsatellite markers in tumor cells indicates a deletion of the allele of the respective gene. Typically, loss of heterozygosity can be demonstrated in hereditary tumor syndromes caused by germline mutations in tumor suppressor genes. The assay is used to **demonstrate the inactivation of both alleles** of the tumor suppressor gene in the tumor tissue. **Microsatellite instability** is typically seen in tumor cells in **Lynch syndrome**. The different length of microsatellite repetitive sequences in tumor tissue compared to normal tissue is due to a defect in DNA repair processes in hereditary mutations of mismatch repair genes.

Minimal Residual Disease

Minimal residual disease (**MRD**) is a condition of **complete clinical remission**, in which the patient cannot demonstrate the minimum number of tumor cells present by conventional methods (eg cytogenetic). The use of molecular-biological methods has significantly **increased the sensitivity of tumor cell detection**. Using **PCR** or **RT-PCR**, it is possible to detect a single tumor cell in a sample with a high number (up to 10^6) of normal cells. In most cases, **high sensitivity** of detection of the target DNA fragment (specific for tumor cells) is achieved by t**wo-step amplification** (nested PCR).

MRD detection by PCR analyzes is used mainly in **hematological malignancies**, possibly aCGH analysis[5]. For example, in **CML**, the **transcript of the *bcr-abl* fusion gene**, which occurs in tumor cells in more than 90% of diseases, can be captured by RT-PCR. A state of "complete molecular remission" in which tumor cells cannot be repeatedly detected by PCR or RT-PCR is considered a favorable prognostic factor. Currently, quantitative PCR methods make it possible to monitor the increase or decrease of surviving tumor cells over time.

References

Related Articles

- Polymerase Chain Reaction
- Mutation Search
- Hereditary Tumor Syndromes
- Possibilities of Detection of Minimal Residual Disease

External Links

- DRÁBEK, Jiří, et al. *Detekce nádorových biomarkerů v molekulárně biologické laboratoři* [online] . 1. vydání. Univerzita Palackého v Olomouci, 2012. 148 s. Dostupné také z <http://detekce.nadorovych.biomarkeru.upol.cz/flipviewerxpress.html>. ISBN 978-80-244-3199-4.

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2. TAVASSOLI, Fattaneh A. a Peter DEVILEE. *Pathology and genetics of tumours of the breast and female genital organs.* 1. vydání. Lyon : IARC Press, 2003. 432 s. World Health Organization classification of tumours; sv. 5. ISBN 92-832-2412-4.
3. HOGERVORST, FB, PM NEDERLOF a JJ GILLE, et al. Large genomic deletions and duplications in the BRCA1 gene identified by a novel quantitative method. *Cancer Res.* 2003, roč. 63, s. 1449-1453, ISSN 0008-5472.
4. STAAF, J, T TÖRNGREN a E RAMBECH, et al. Detection and precise mapping of germline rearrangements in BRCA1, BRCA2, MSH2, and MLH1 using zoom-in array comparative genomic hybridization (aCGH). *Hum Mutat.* 2008, roč. 29, s. 555-564, ISSN 1059-7794.
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