Molecular pathology in routine diagnostics of solid tumors

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A. Types of molecular markers
B. Different molecular tools
C. Clinical utility of tumor markers in oncology: examples of molecular testing in routine daily practice

Types of molecular markers

- DIAGNOSTIC markers
- PROGNOSTIC markers
- PREDICTIVE markers

Diagnostic markers

- Large category of molecular tests aid in the diagnosis or subclassification of a particular disease state
- Diagnostic subclassification may result in different management of the disease especially in soft tissue pathology and hematopathology
  - MCM2 and CDK4 amplification in well-differentiated and dedifferentiated liposarcomas
  - MYC amplification in patanodermal angiomyxoma
  - Rearrangement of CHK1, CHK2 and SYT genes in mixed liposarcoma,
  - Ewing sarcoma and synovial sarcoma respectively
  - KIT and PDGFRA mutation in Gastrointestinal stromal tumors (GIST)
  - Rearrangement of R13,2 gene in follicular lymphoma
  - Rearrangement of MYC gene in Burkitt lymphoma
  

Prognostic markers

- Prognostic markers have an association with some clinical outcomes (overall survival, recurrence-free survival), independent of the treatment rendered
- e.g., presence of p53 mutations, which identify subsets of patients who will have a more aggressive disease course for certain cancers, regardless of current treatment options

Predictive markers

- Predictive markers predict the activity of a specific class or type of therapy, and are used to help make more specific treatment decisions
- They are used as indicators of the likely benefit of a specific treatment to a specific patient ("personalized medicine")
- e.g., HER2 status in breast and gastric cancer: HER2-positivity (amplification) is predictive of potential trastuzumab response
- e.g., BRAF V600E mutation is predictive of sensitivity to vemurafenib
Molecular diagnostic tools

- conventional cytogenetic/chromosome analysis
  short-term, primary cultures followed by karyotyping

- Molecular cytogenetics
  *fluorescence in situ hybridization (FISH)*: detecting of cancer-related translocations, deletions and amplifications
  *molecular karyotyping (cytogenomic arrays) (CGH array, SNP analysis)*: detecting whole genome copy number changes including losses, gains and amplifications

Conventional karyotyping

- **Advantages:**
  - global genetic information in single assay
  - variants uncovered (undetectable by FISH and RT-PCR)
  - diagnostically useful: sensitive, specific
  - provides direction for further molecular studies

- **Limitations:**
  - requires fresh tissue
  - most cell culture 1-10 days
  - complex karyotypes, suboptimal morphology
  - normal karyotypes (overgrowth normal fibroblasts, infiltrating cells)

Multiplex Ligation-dependent Probe Amplification

- Special form of multiplex PCR
- Detection of aberrant copy number of 55 genomic DNA sequences in a single, PCR-based, reaction
- MPRA can also be used for detecting methylation status and known point mutations
- Amplification of MLPA probes, not sample DNA
- Requires a minimum of 20ng of human DNA (5000 cells)
- MPRA can also be used on partially degraded DNA (e.g. FFPE)


Hörmig-Klett C and Savola S. Diag Nol Pathol 2012;21(4):189-205

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Advantages of MLPA for tumor applications

- MLPA is excellent technique for large scale validation of new biomarkers and for use in routine diagnostics
- Probe for both methylation and copy number changes and a few probes for specific point mutations can be combined in one test
- High throughput: up to 96 samples can be analyzed in 24h, with little hands on time; 55 targets can be analyzed simultaneously!

Limitations of MLPA for tumor applications

- MLPA detects in RELATIVE amounts of target sequences: gains/losses may not be detected if percentage of tumor cells is too low or due subclonality (<20-30%)
- NO MORPHOLOGICAL CORRELATION
- Selection of reference probes for MLPA mix is critical: they should locate on "silent regions" of the tumor genome
- MLPA technology is not suitable for the detection of unknown mutations

MLPA

- Copy number detection: MYCN (neuroblastoma), HER2 (breast cancer, gastric cancer), MDM2 (liposarcoma), EGFR (glioblastoma)
- Point mutation detection with MLPA: IDH1/2 (diffuse glioma), BRAF (melanoma, pilocytic astrocytoma), EGFR (lung carcinoma)

*genes with less-defined hot spots or many point mutations within a short distance from each other (TP53, p16CDK4/9/2A), are less suitable for the design of mutation-specific MLPA probe
Molecular pathology: routine daily practice

- **In situ hybridization techniques**
  - fluorescence in situ hybridization (FISH)
  - chromogenic in situ hybridization (CISH)
  - silver in situ hybridization (SISH)

- **Polymerase chain reaction (PCR), reverse transcription-polymerase chain reaction (RT-PCR)**

In situ hybridization: applications in molecular diagnostics

- Numerical chromosomal alterations
- Gene amplifications and deletions
- Chromosomal translocations (lymphomas and sarcomas)
- Virus detection (DNA or RNA) (HPV, EBV)
**FISH: advantages**

- Fresh, frozen, paraffin-embedde material
- Localize alteration (amplification, translocation) in specific cells an tissue types
- Useful if tumor is heterogeneous
- Diagnostically useful: fine needle biopsy, sensitive, specific
- Can provide results if karyotyping or RT-PCR is inconclusive
- Rapid turn-around time
- Validation and implementation easy
- Normal tissue parts can serve as FISH control

**FISH: limitations**

- Relatively gross approach (no information on fusion genes and variants)
- Number of commercially available probes is limited (Abbott Molecular, Kreatech)
- Requires fluorescence microscope
- Interpretation may be challenging, expertise required
- Period of storage