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

-*MDM2* and *CDK4* amplification in well-differentiated and dedifferentiated liposarcomas
-*MYC* amplification in postradiation angiosarcoma
-rearrangement of *CHOP*, *EWS* and *SYT* genes in myxoid liposarcoma, Ewing sarcoma and synovial sarcoma respectively
-*KIT* and *PDGFRα* mutation in Gastrointestinal stromal tumors (GIST)
-rearrangement of *BCL2* 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 og sensitivity to vemurafinib

Molecular diagnostic tools

- **conventional cytogenetic/chromosome analysis**

  short-term, primary cultures followed by karyotyping

Conventional karyotyping

- **Advantages:**
  -global genetic infomration 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
  -mostly cell culture 1-10 days
  -complex karyotypes, suboptimal morphology
  -normal karyotypes (overgrowth normal fibroblasts, infiltrating cells)

Molecular diagnostic tools

- **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

Molecular diagnostic tools

- **Molecular assays**
  * *Polymerase chain reaction (PCR), reverse transcription-polymerase chain reaction (RT-PCR):* assays for known mutations, translocations
  * *Multiplex Ligation-dependent Probe Amplification (MLPA):* detecting losses, gains and amplifications
  * *Expression profiling (cDNA arrays)*
  * *Next generation whole genome technologies (NGS)*

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
- MLPA 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 (3000 cells)
- MLPA can also be used on partially degraded DNA (e.g. **FFPE**)

Hömig-Hölzel C and Savola S. Diagn Mol Pathol 2012;21(4):189-206
Creytens D, van Gorp J, Ferdinande L, Speel EJ and Libbrecht L. Diagn Mol Pathol 2014
Creytens D, van Gorp J, Savola S, Ferdinande L, Mentzel T and Libbrecht L. Virchows Arch 2014;465(1):97-108
Creytens D, van Gorp J, Speel EJ and Ferdinande L. Anticancer Res 2014

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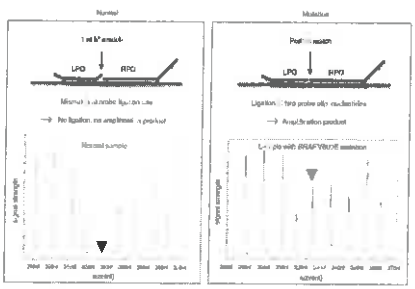

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Advantages of MLPA for tumor applications

- 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!

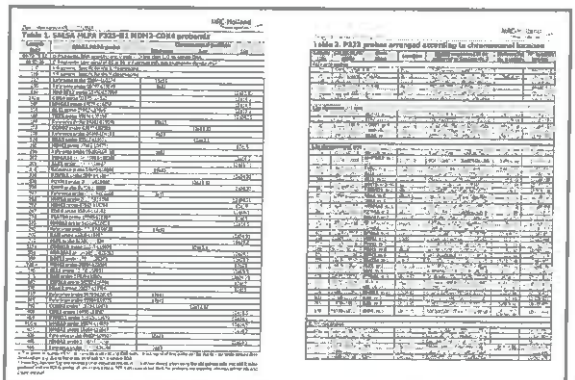
Limitations of MLPA for tumor applications

- 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, p16/CDKN2A), are less suitable for the design of mutation-specific MLPA probes!



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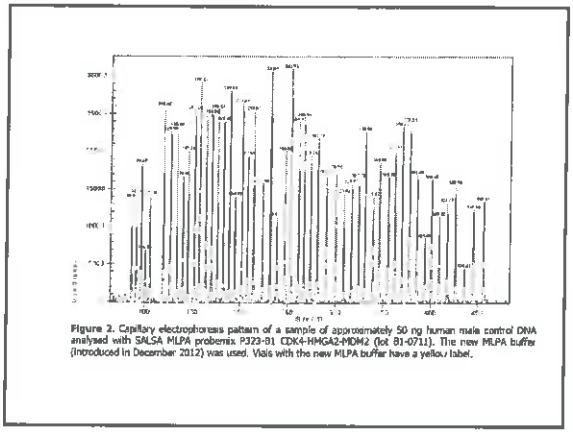
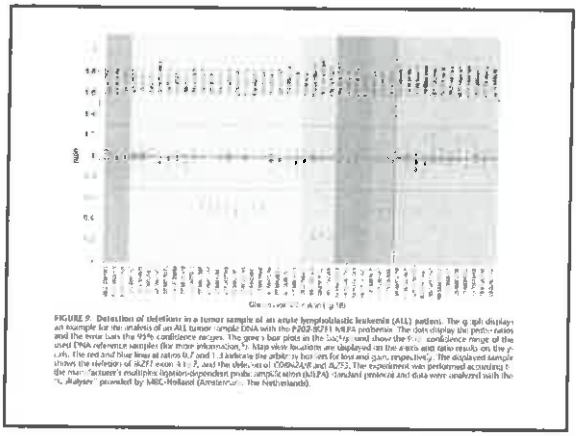


Figure 2. Capillary electrophoresis pattern of a sample of approximately 50 ng human male control DNA analysed with SALSA MLPA probemix P323-B1 CDK4-HMGA2-MDM2 (lot B1-0711). The new MLPA buffer (introduced in December 2012) was used. Vials with the new MLPA buffer have a yellow label.



Molecular pathology: routine daily practice

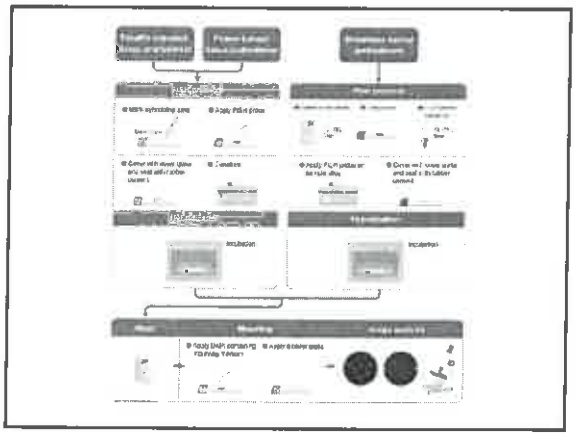
- ***In situ hybridization techniques***

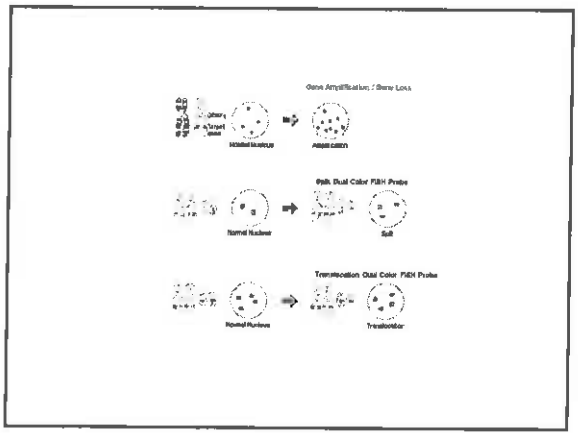
*fluorescence in situ hybridization (**FISH**)
*chromogenic in situ hybridization (**CISH**)
*silver in situ hybridization (**SISH**)

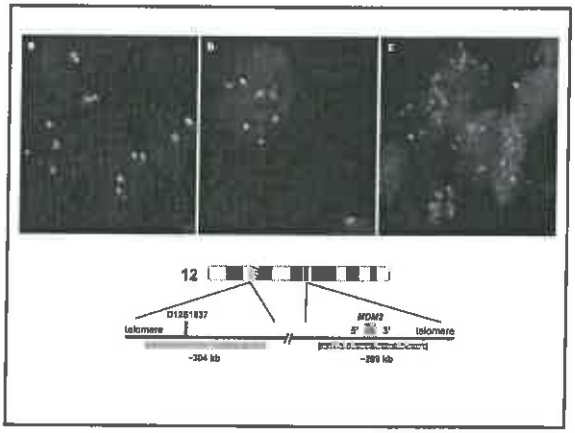
- *Polymerase chain reaction (**PCR**), reverse transcription-polymerase chain reaction (**RT-PCR**):*

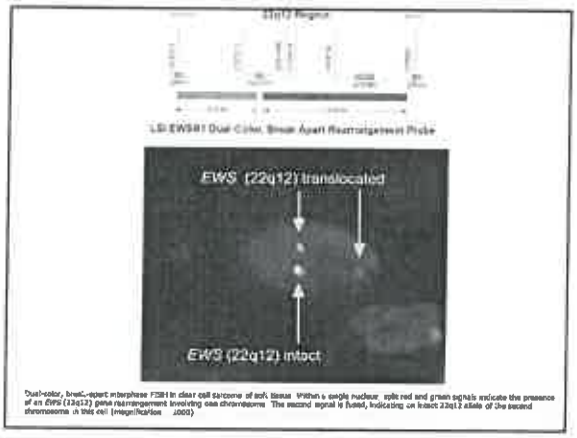
In situ hybridization: applications in molecular diagnostics

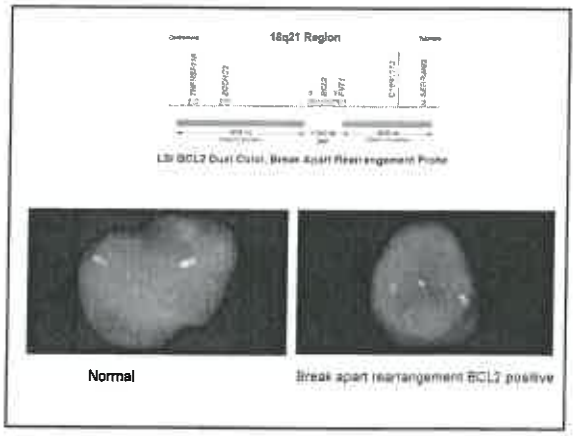
- Numerical chromosomal alterations
- Gene amplifications and deletions
- Chromosomal translocations (lymphomas and sarcomas)
- Virus detection (DNA or RNA) (HPV, EBV)











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 implemenation 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