Classical and molecular cytogenetics in oncology

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Cytogenetic examination

Clinical cytogenetics → determination of karyotype of patients with inborn defects

Prenatal diagnostics → examination of chromosomal complement of embryos, in vitro fertilization

Oncocytogenetics → specification of diagnosis and prognosis of malignant diseases

Services of hygiene laboratories - testing of mutagenicity of chemicals on human organism on chromosomal level, radiation cytogenetics etc.
Chromosomal aberrations

Changes of number or structure of chromosomes

I. Inborn (constitutional) aberrations are fundamental for origin of syndromes due to anomalies of chromosomes (for example Down’s sy); changes of chromosomes are present in all cells of the body.

II. Acquired changes of chromosomes in malignant cells; they have clonal character (only certain cellular clones are involved).
Cancer cells

- high genome instability - one of the most important events in the malignant process
- gene mutations and numerical and/or structural chromosomal aberrations

Nonrandom chromosomal aberrations are associated with specific disease subtypes and have a clear prognostic implications.
Methods

Conventional cytogenetic analysis

I-FISH

WCP-FISH

mFISH

mBAND

CGH

Array CGH
Conventional cytogenetic analysis

Karyotype analysis
Detection of chromosomal aberrations
(numerical x structural)
medium kolchicin hypotonie fixace preparace

inkubace při 37 °C

Postup při kultivaci a přípravě preparátů k cytogenetickému vyšetření
Limitations of classical cytogenetics

- Sensitivity of chromosomal banding techniques is limited.
- These techniques require a high rate of dividing cells with good chromosomal morphology (resolution limit of 10 Mb).
- In some leukemias, malignant cells are not proliferating in the cell culture (only the normal cells are dividing) → the results of cytogenetic examination is not representative for malignant process.
- Chromosomal changes in leukemic cells are very often complex or could be cryptic under the limit of light microscopy.
Fluorescence *in situ* hybridization (FISH)

- permits detection of selected acquired genetic changes in dividing (metaphase) and nondividing (interphase nuclei) cells

- is useful in establishing the percentage of neoplastic cells at the time of diagnosis and after therapy

- FISH studies are used to investigate the origin and progression of hematologic malignancies and to establish which hematopoietic compartments are involved in neoplastic processes
Fluorescence in situ hybridization (FISH)

Cytogenetický preparát

Denaturace buněčné DNA fixované na cytogenetickém preparátu

Dvouřetězcová značená DNA

Denaturace DNA sondy

Hybridizace DNA sondy ke komplementárním úsekům cílové DNA fixované na cytogenetickém preparátu

Analýza fluorescenčních signálů ve fluorescenčním mikroskopu
Probes for specific chromosomal structures:

α-satellite DNA - centromeres

Determination of numerical aberrations, identification of the origin of centromeres in marker-chromosomes, specification of cells after bone marrow transplantation (opposite sex of donor and recipient)

metaphase x interphase
Locus specific DNA probes:

- Mapping of genes on chromosomes, detection of structural rearrangements (translocations, deletions)

metaphase x interphase
Chromosome painting probes:

They contain sequences from whole chromosomes or chromosomal parts (partial probes).

- Determination of structural rearrangements (translocations and deletions of large extent), identification of origin of marker-chromosomes

Metaphase only
Multicolor FISH - mFISH

allows in one hybridization experiment distinguish according to different color every pair of autosomes and sex chromosomes and then it is possible to make analyses of the whole genome and every structural and numerical rearrangement.
analyses of complex chromosomal rearrangements in bone marrow cells of patients with hematological malignancies will bring us detailed informations about involvement of specific chromosomes or their regions into rearrangements.
Multicolor banding with high resolution - mBAND

- enables determination of exact breakpoints of chromosomal aberrations with much higher resolution than classical banding

mBAND 1

\[ \text{der}(1)(1;11)(pter\rightarrow q31::q22.2q23.3) \]

mBAND 11

\[ \text{der}(1)(1;11)(pter\rightarrow q31::q22.2q23.3) \]
Array-based comparative genomic hybridization (aCGH)

- new tool to search for recurrent gains or loss of chromosomal regions throughout the genome according to detection with very high resolution of copy number changes at DNA level

- only recently is aCGH successfully utilised in diagnostics of leukemias and the results revealed a large spectrum of genomic imbalances, including novel recurrent deletions and amplifications

BAC arrays ~1MB
Oligo arrays ~100 kb (maximal resolution ~ 35 kb)
The impact of conventional and molecular cytogenetic analysis in oncohematology

- Is part of the work up at diagnosis
- Provides comprehensive information on the karyotype
  - help to specify diagnosis
  - help to determine the prognosis
  - help monitor effectiveness of treatment
Hematological malignancies

- Chronic Myeloid Leukemia (CML)
- Acute Myeloid Leukemia (AML)
- Acute Lymphoblastic Leukemia (ALL)
- Myelodysplastic Syndromes (MDS)
- Chronic Lymphocytic Leukemia (CLL)
- Multiple Myeloma (MM)
- Non Hodgkin Lymphoma (NHL)

Recommended examination procedures - depending on the type of disease and treatment protocol
**Chronic Myeloid Leukemia (CML)**

- 15-20% of all leukemia cases
- Mainly in adults (median age 65 years)
- Tri-phasic disease: **CP** - chronic phase (relatively benign, 3-9 years)
  - **AP** - More malignant accelerated phase
  - **BC** - Terminal blast crisis
- Blasts increase rapidly (crowd out healthy cells)
- One of the best-studied malignancies - has served as a paradigm for elucidation how genetic changes cause cells to become malignant: Ph chromosome \( t(9;22)(q34;q11) \) - BCR/ABL fusion
- One of the first malignancies in which a therapy targeting the underlying molecular defect has improved the clinical outcome of patients: Gleevec™ (Novartis) used for therapy
- \( t(9;22)(q34;q11) \) in 90-95% of patients (detectable by \( CC \)) - BCR/ABL fusion gene
Chronic Myeloid Leukemia (CML)

Conventional Cytogenetics:

BM
24h/48h cultivation (adults)
at least 20 metaphases

- normal karyotype or insufficient metaphases or normal karyotype without t(9;22)
  - FISH: BCR/ABL1

- aberrant karyotype
  - targeted FISH or other molecular cytogenetic techniques (mFISH/mBAND, array CGH)

Report according to ISCN
Acute Myeloid Leukemia (AML)

- Heterogenous group of malignant diseases of haemopoiesis
- Accumulation of immature myeloid cells (myeloblasts) in bone marrow
- Diagnosed in all age groups
- Most commonly affects people older than 60 years (median age 64-68 years)
- Secondary AML, therapy-related AML
- Aggressive disease - median OS 2-3 months
- Specific chromosomal aberrations with clear prognostic significance → stratification of therapy according to cytogenetic findings
## Cytogenetic findings in AML

<table>
<thead>
<tr>
<th>Chromosomal aberration</th>
<th>Genes</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(8;21)(q22;q22)</td>
<td>RUNX1-RUNX1T1</td>
<td>good</td>
</tr>
<tr>
<td>inv(16)(p13.1q22) or t(16;16)(p13.1;q22)</td>
<td>CBFB-MYH11</td>
<td>good</td>
</tr>
<tr>
<td>t(15;17)(q22;q12)</td>
<td>PML-RARA</td>
<td>good</td>
</tr>
<tr>
<td>t(9;11)(p22;q23)</td>
<td>MLLT3-MLL</td>
<td>intermedial</td>
</tr>
<tr>
<td>t(6;9)(p23;q34)</td>
<td>DEK-NUP214</td>
<td>poor</td>
</tr>
<tr>
<td>inv(3)(q21q26.2) or t(3;3)(q21;q26.2)</td>
<td>RPN1-EVI1</td>
<td>worst</td>
</tr>
<tr>
<td>t(1;22)(p13;q13)</td>
<td>RBM15-MKL1</td>
<td>good</td>
</tr>
<tr>
<td>Rearrangements of MLL gene</td>
<td>MLL</td>
<td>poor</td>
</tr>
<tr>
<td>monosomy 7 or deletion of 7q31</td>
<td></td>
<td>poor</td>
</tr>
<tr>
<td>deletion of 5q31</td>
<td>?</td>
<td>poor</td>
</tr>
<tr>
<td>complex chromosomal aberrations</td>
<td>?</td>
<td>worst</td>
</tr>
</tbody>
</table>
Prognostic impact of specific chromosomal aberrations in acute myeloid leukemia (AML)
Acute Myeloid Leukemia (AML)

Conventional Cytogenetics:
BM
24h/48h cultivation (adults)
at least 20 metaphases

- normal karyotype or insufficient metaphases
- aberrant karyotype

Subtype specific FISH:
- MLL, 5q31/5p15, 7q31/7, 8/9
- PML/RARA, AML1/ETO, CBFB

Targeted FISH or other molecular cytogenetic techniques (mFISH/mBAND, array CGH)

Report according to ISCN
Acute Lymphoblastic Leukemia (ALL) Overview

- Accumulation of malignant, immature lymphoid cells in the bone marrow and mostly also in peripheral blood
- Most common malignancy in children – representing 25% of pediatric cancer
- 80% of pediatric leukemias
- Heterogenous disease - distinct therapeutic and prognostic implications
- Chromosomal aberrations:
  - found up to 90% of cases
  - one of the most important prognostic factors!!
- Conventional chromosomal analysis are limited by the low mitotic activity of malignant B-lymphocytes → I-FISH for the most common recurrent chromosomal aberrations
## Cytogenetic findings in ALL

<table>
<thead>
<tr>
<th>Risk Group</th>
<th>Cytogenetic finding</th>
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<tbody>
<tr>
<td><strong>Low risk</strong></td>
<td>High hyperdiploidy (51-65 chromosomes)</td>
</tr>
<tr>
<td></td>
<td><em>ETV6-RUNX1</em></td>
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<tr>
<td><strong>Standard risk</strong></td>
<td>t(1;19)(q23;p13)</td>
</tr>
<tr>
<td></td>
<td><em>IGH-CEBP</em></td>
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<td></td>
<td><em>IGH-ID4</em></td>
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<td></td>
<td>del(6)(q)</td>
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<tr>
<td></td>
<td>Aberration of 9p</td>
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<tr>
<td></td>
<td>Aberration of 11q</td>
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<tr>
<td></td>
<td>dup(1q)</td>
</tr>
<tr>
<td></td>
<td>-7</td>
</tr>
<tr>
<td></td>
<td>dic(9;20)(p13;q11)</td>
</tr>
<tr>
<td></td>
<td>dic(9;12)(p11-21;p11-13)</td>
</tr>
<tr>
<td></td>
<td>Any other aberration</td>
</tr>
<tr>
<td></td>
<td>normal karyotype</td>
</tr>
<tr>
<td><strong>High risk</strong></td>
<td>t(9;22)(q34:q11)</td>
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<tr>
<td></td>
<td>iAMP21</td>
</tr>
<tr>
<td></td>
<td><em>MLL</em> translocations</td>
</tr>
<tr>
<td></td>
<td>„near“ haploidy (&lt;30 chromosomes)</td>
</tr>
<tr>
<td></td>
<td>Low hypodiploidy (30-39 chromosomes)</td>
</tr>
<tr>
<td></td>
<td>t(17;19)(q23;p13)</td>
</tr>
<tr>
<td></td>
<td>Aberration of 17p</td>
</tr>
<tr>
<td></td>
<td>Loss of 13q</td>
</tr>
</tbody>
</table>

*Moorman et al., Lancet Oncol 2010*
Today, karyotype remains the gold standard for classification of patients with childhood ALL into risk group for treatment.
Acute Lymphoblastic Leukemia (ALL)

Conventional Cytogenetics:
- BM direct/24h cultivation (childhood)
- 24h/48h cultivation (adults)
- at least 20 metaphases

B-ALL:
- "triple test"
  - ETV6/RUNX1
  - MLL
  - Hyperdiploidy

T-ALL:
- TCR genes
  - TCRαδ (14q11), TCRβ (7q34), TCRγ (7p14)
  - TP16 (9p21)
  - ABL1 (9q34)

I-FISH:
- ETV6/RUNX1
- MLL

or

aberrant karyotype

targeted FISH or other molecular cytogenetic techniques (mFISH/mBAND, array CGH)

Report according to ISCN
CONCLUSIONS

- The impact of cytogenetic analyses on clinical diagnostics of hematological malignancies has increased dramatically during recent years → laboratory techniques have to be optimized to provide reliable results for optimal patient care.

- Quick and correct results save time and money by preventing unnecessary additional diagnostics and suboptimal treatment approaches.

- Standardization of cytogenetic diagnostic protocols may help to improve diagnosis, and hence treatment outcome of hematologic malignancies.