CHROMOSOME ABNORMALITIES IN HUMAN CANCER

K. Michalová, Z. Zemanová

3rd Medical Department of General Faculty Hospital and 1st Medical Faculty of Charles University, U nemocnice 1, 128 08 Prague, Czech Republic, Phone: +420 2 24962927, Fax: +420 2 296872, e-mail: kyra@vfni.cz

ABSTRACT: The karyotypic changes in malignant tumor cells are unevenly distributed throughout the human genome. Modern cancer cytogenetics showed that different chromosomal bands are preferentially involved in rearrangements in different neoplasms and specific aberrations were identified. Due to the availability of bone marrow cells first insights were done into pathogenesis of hematologic malignancies and were accompanied by elucidation of the role of chromosomal translocations and deletions. Deletions result very often in loss of a tumor suppressor genes whereas specific translocations and inversions lead to the two principal consequences: 1. new fusion gene encoding chimeric protein is created-mostly in myeloid disorders, 2. gene for the immunoglobulin or T-cell receptor is moved near to the proto-oncogene and enhances its activity – mostly for lymphoid disorders. All three above mentioned rearrangements were later on proved in solid tumors as well. The breakpoints of many translocations specific for different hematologic and solid tumors have been cloned and serve as molecular markers for diagnosis. Cytogenetic analyses are part of the routine work-out of the patients. A variety of molecular techniques are now available for wide genome screening of alterations in copy number, structure and expression of genes and DNA sequences. Molecular cytogenetics has special methods: fluorescence in situ hybridization (FISH), comparative genomic hybridization (CGH), spectral karyotyping (SKY) and multicolor FISH (mFISH). Except for the basic research of human neoplasias all these methods are used routinely to monitor the effect of the treatment and follow the residual tumor cells after chemotherapy and/or bone marrow transplantation.

INTRODUCTION

The roots of onc cytogenetics can be found in the last years of the 19th century at the time when Arnold (1879) described mitotic errors in tumor cells. However, as a real founder of tumor cytogenetics is considered Boveri, who published in 1914 the first chromosomal hypothesis of origin of malignant tumors. Boveri suggested that tumor cells are originally normal cells and their abnormal behavior lies inside of the cells and not in their surrounding. The content of chromatin in tumor cells is abnormal and the normal cellular properties are lost. At that time the quality of the cytological techniques did not enable to test this hypothesis and actually it took more than 40 years before the exact number of human chromosomes was established by Tjio and Levan (1956) and clinical cytogenetics became an important part of pre- and postnatal genetic counselling.

Oncocytogenetics is independent part of the medical genetics concerned with acquired chromosomal changes in benign and malignant tumor cells. Aberrant karyotype was found first in hematologic malignancies, and later on in solid tumors in more than 50% of specimens of bone marrow cells and/or peripheral lymphocytes studied by classical cytogenetic techniques. The proof of existence of pathological clone in examined sample is according to Heim and Mitelman (1995) the presence of two mitoses with the same chromosomal rearrangement or extra copy of the same chromosome, or three mitoses with the same chromosomal loss.

CHROMOSOMAL ABERRATIONS IN NEOPLASTIC TUMORS

The chromosomal changes can be numerical and/or structural ones and are specific for certain type or subtype of the tumor. Cytogenetic findings in different types of tumors serve not only to the basic research of pathogenesis of neoplastic process but are routinely used by clinicians to set up precise diagnosis of the patients, specially in hematologic diseases.

Partially supported by the Internal Grant Agency of Ministry of Health of Czech Republic (Nos. NC 5010-3 and NE 4744-3), Grant Agency of Czech Republic (Grant No.302/98/0071) and Internal Grant for Development of Ministry of Education and Sports of Czech Republic (No. CJ 13/98 111100004).
Clinical applications of cytogenetic examinations are as follows:
1. Detection of numerical and structural chromosomal abnormalities
2. Identification of marker chromosomes
3. Monitoring of the effects of the therapy and detection of minimal residual disease
4. Detection of early relapse
5. Identification of the origin of bone marrow cells following bone marrow transplantation
6. Examination of the karyotypic pattern of non-dividing or interphase cells
7. Detection of gene amplification
8. Prognosis of the individual patients

Chromosomal translocations

Consistent and specific translocations and inversions are involved in tumor aetiology activating proto-oncogenes at or near the chromosomal breakpoints (Tab. I). Genes, localized at the specific breakpoints are studied by molecular biology methods. These genes are cloned and sequenced and their functions and products are studied from the point of view of their role in the pathogenesis of malignant disease. As an example can be quoted extensive research which was done by analysing the specific translocations in leukaemias. The typical representative of specific translocation which is present in 95% of patients with chronic myeloid leukemia (CML) is so called Philadelphia chromosome (Ph chromosome). It is reciprocal translocation between long arms of chromosome 9 and 22 – t(9;22)(q34;q11). Schematic representation of t(9;22) is in Fig. 1 with marked localization of the genes which are moved during the translocation event.

Two types of translocations were described in hematologic malignancies. The first one is leading to the occurrence of new, fused, hybrid gene coding product which differ somewhat from the original one, while the result of the second type is the displacement of the genes, mainly oncogenes, close to the immunoglobulin genes and enhancement of their functions. Both types of translocations play important role in malignant formation of the cells (Rabbits, 1994). It is hypothesized that the same mechanisms exist in solid tumors and that there are not more then these two types of translocations. Translocation t(9;22)(q34;q11) belongs to the first type and new hybrid gene is coding the protein with different tyrosine kinase activity.

1. Breakpoints of the most frequent specific chromosomal translocations, affected genes and diseases with this type of chromosomal rearrangements

<table>
<thead>
<tr>
<th>Fused genes – hematopoietic tumors</th>
<th>t(9;22)(q34;q11)</th>
<th>c-ABL/BCR</th>
<th>CML, ALL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t(15;17)(q21q11)</td>
<td>PML/RARA</td>
<td>APL</td>
</tr>
<tr>
<td></td>
<td>t(8;21)(q22;q22)</td>
<td>AML1/ETO</td>
<td>AML-M2</td>
</tr>
<tr>
<td></td>
<td>t(4;11)(q21;q23)</td>
<td>MLL/AF4</td>
<td>ALL, pro-B ALL, AML</td>
</tr>
<tr>
<td></td>
<td>t(11;19)(q23p13.3)</td>
<td>PBX1/E2A</td>
<td>Pre-B ALL</td>
</tr>
<tr>
<td></td>
<td>t(12;21)(p13;q22)</td>
<td>TEL/AML1</td>
<td>Children pre-B ALL</td>
</tr>
<tr>
<td></td>
<td>inv(16)(p13q22)</td>
<td>CBFI/SMMHC</td>
<td>AMMOL</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fused genes – solid tumors</th>
<th>t(11;22)(q24q12)</th>
<th>FLI/EWS</th>
<th>Ewing's sarcoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(12;22)(q13q12)</td>
<td>ERO/EWS</td>
<td>Ewing's sarcoma</td>
<td></td>
</tr>
<tr>
<td>t(12;16)(q13p11)</td>
<td>CHO/PFUS</td>
<td>Liposarcoma</td>
<td></td>
</tr>
<tr>
<td>t(2;13)(q35q14)</td>
<td>PAX3/FKHR</td>
<td>Rhabdomyosarcoma</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Non-fusions – hematopoietic tumors</th>
<th>t(8;14)(q24q32)</th>
<th>c-MYC (8q24)</th>
<th>BL, BL-ALL</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(8;14)(q24q11)</td>
<td>c-MYC (8q24)</td>
<td>T-ALL</td>
<td></td>
</tr>
<tr>
<td>t(8;12)(q24q22)</td>
<td>c-MYC (8q24)</td>
<td>B-CLL, ALL</td>
<td></td>
</tr>
<tr>
<td>t(11;14)(q32q21)</td>
<td>BCL-2 (18q21)</td>
<td>FL</td>
<td></td>
</tr>
<tr>
<td>t(11;14)(q13q32)</td>
<td>BCL1/PRAD1</td>
<td>B-CLL and others</td>
<td></td>
</tr>
</tbody>
</table>


VET. MED. – CZECH, 45, 2000 (10–11): 331–335
2. Diagramatic presentation of interstitial deletions of chromosome 5 found in patients with myelodysplastic syndrome

Chromosomal deletions

Deletions are specific acquired rearrangements which are connected with the loss of chromosomal material. Most of the deletions are interstitial and we can find them in the tissue of solid tumors, but they were described in hematologic malignancies as well. The first were cytogenetically and molecularly studied three solid tumors of childhood – retinoblastoma, neuroblastoma and Wilms tumor. Retinoblastoma tumors may develop sporadically or are hereditary and one or both eyes can be affected. Retinoblastoma has served as an example to elucidate the role of tumor suppressor genes in carcinogenesis. Retinoblastoma gene (Rb gene) which was cloned in 1986 was the first known tumor suppressor gene and its loss was proved in tumor tissue. Retinoblastoma became a model for extensive studies of tumorogenesis (Horstemke, 1992; Heim and Mitelman, 1995). Since that time the loss of tumor suppressor genes was determined in many solid tumors and hematologic malignancies and the significance of deletions is studied on molecular level. As one of many examples the loss of p53 gene in solid and hematologic malignant tumors can be presented. For preleukemias, the interstitial deletion of long arms of chromosome 5 was described by van den Berghe in (1985) and 5q- syndrome has become a special clinical entity (Fig. 2). Many other malignancies with specific acquired deletions were described and these findings are routinely used for diagnostic work. But there is still necessary to perform cytogenetic studies in large cohort of patients with certain type of malignancy and specific acquired chromosomal deletions to elucidate significance of these changes for course of the disease and response to the therapy.

Numerical chromosomal changes

Numerical chromosomal aberrations i.e. gain or the loss of the whole chromosome are trisomies and monosomies which can be found in different tumors. By many cytogenetic studies it was proved that every numerical change may be found-even as the sole anomaly in almost every neoplastic disorder (van den Berghe, 1999). During the last years numerical chromosomal changes were found in benign tumors as for example trisomy 7 in tumors of the digestive tract (Heim and Mitelman, 1995) and consistent monosomies and trisomies were found in hematologic neoplasias. Pathogenetic significance of such abnormalities is totally unknown. Diagnostic and prognostic value of different aberrations, determined by classical chromosome banding techniques is now well established, mainly for hematologic tumors.

MOLECULAR CYTOGENETICS

Main progress in oncogenicetics brought molecular cytogenetic techniques. Different modifications of fluorescence in situ hybridization (FISH) were introduced into routine practice and new, specific chromosomal changes were found. As an example we present the reciprocal translocation t(12;21)(p13;q22) which can be found in bone marrow cells of almost 30% of children with B-cell acute lymphocytic leukemia (B-ALL). This translocation is not detectable by G-banding, because involved parts of the short arms of chromosome 12 and long arms of chromosome 21 are approximately of the same size and color intensity after G-banding (Fig. 3). Translocation t(12;21) was found using double color FISH method (Romana et al., 1995). Now the molecular basis of this translocation is known (fusion of TEL

3. Reciprocal translocation between chromosomes 12 and 21. Fused gene AML1/TEL is located on derivative chromosome 21

VET. MED. – CZECH, 45, 2000 (10–11): 331–335 333
and AML1 genes) and all children with acute lympho-
cytic leukemia can be screened by PCR for presence of
t(12;21). However, cytogenetic examinations at diagno-
sis and during the treatment are necessary as well, be-
cause additional chromosomal changes can be found.
As one of the most frequent trisomy 21 is present in pa-
tients bone marrow cells. According to the results of
extensive European study it seems that TEL/AML1
translocation, unlike many others, gives good progno-
sis to the patients. We participate in the prospective stu-
dy in Czech Republic, where the treatment and course
of the disease is evaluated in children which have
t(12;21) as a single change in the karyotype in compa-
rison to those who have additional complex chromoso-
mal rearrangements (Zemanova et al., 1999).

Moreover, a variety of techniques are now available
for wide genome screening of alterations in copy num-
ber, structure and expression of genes and DNA se-
quences. Molecular cytogenetics has special techniques
of comparative genomic hybridization (CGH), spectral
caryotyping (SKY) and multicolor FISH (mFISH)
(Schröck et al., 1996; Speicher et al., 1996; Ried et al.,
1997). SKY and mFISH are the newest modifications
of the hybridization techniques. These techniques allow
in one hybridization experiment to color all human
chromosomes and identify chromosomal rearrange-
ments. These both methods are above all suitable to
identify even cryptic chromosomal translocations and
insertions, which are not resolved by classical cytoge-
netics (Le Beau, 1996). Double color FISH, which is
currently used in most laboratories, is suitable to con-
firm chromosomal changes which are suspected on the
basis of conventional cytogenetic analyses. Small rea-
marrangements, specially the reciprocal translocations and
insertions, are not discovered by G-banding despite the
fact that the quality of classical chromosomal prepara-
tions has improved tremendously during the last decade
and practically reached the resolution limit of light
microscopy. However, the identification of such chan-
ges is of great importance both in the analysis of somatic
cells of patients with congenital syndromes as well as
in malignant tumor cells.

In malignant tumors, where the acquired chromoso-
mal aberrations were found in almost 75% of patients,
we can expect to ascertain by mFISH new recurrent
chromosomal changes which could play role for clin-
ical and molecular biological diagnosis (Heim and Mit-
telman, 1995; Le Beau, 1996). Non-random chromoso-
mal changes in malignancies often mirror events at the
molecular level and provide entry points for gene iden-
tification strategy (Bishop, 1987; Ried et al., 1997;
Gilliland, 1998) and contribute further to the under-
standing of malignant cell transformation.

As mFISH adds another level of resolution, the possi-
bility of existence of malignant cells with normal kary-
otype at this level should be re-evaluated. So far only
few patients with cancer and normal karyotype estab-
lished by classical cytogenetics were examined. Beverloo
et al. (1999) published results obtained by examination
of 20 patients with acute myeloid leukemia (AML) and
normal classical cytogenetic findings, that were also
examined by SKY method of multicolor FISH. The au-
thors did not find the unusual chromosomal changes in
bone marrow cells of these patients, but discovered
cryptic rearrangements, not identifiable by classical
G-banding in patients with complex karyotype. There-
fore they expect more chromosomal changes in patients
with advanced malignancy. Their preliminary results
support our findings in patients with advanced myelo-
dysplastic syndrome and deletions of 5q (Michalová et
al., 1999). The deletion of 5q31 is typical chromosomal
change in patients with myelodysplastic syndromes
(MDS). The clone with deletion can be found at the
time of diagnosis and later, during the progression of
the disease, further clonal evolution towards numerical
and structural aberrations of chromosomes can be seen.
During the last four years we concentrated on analyses
of instability of deleted chromosome 5 in patients with
advanced forms of MDS. We have found that deleted
chromosome 5 is involved in many complex rearrange-
ments with different partner chromosomes for translo-
cated parts of 5q deleted chromosome

CONCLUSIONS

Cytogenetic research as well as clinical oncocyto-
genetics progressed remarkably during the last few years.
The advances in generation of DNA probes, in situ
hybridization technology, fluorescence microscopy and
fast progress in digital and spectral imaging gave the
chance to study precisely genetic rearrangements in
malignant tumors. Many findings of previous research
will be reviewed by molecular cytogenetic techniques.
The application of new techniques in human tumors ad-
ded significant amount to our knowledge of non-ran-
dom tumor specific genetic changes and their signifi-
cance in cancerogenesis. Clinical applications of
cytogenetic results are routinely used to improve diag-
nostic and can help to devise therapeutic regimens.

REFERENCES

Arnold (1879): cited in Gebhardt E.(1987): Cytogenetic stud-
ies in human neoplasia. In: Obo G., Basler A. (eds.): Cyto-

Beverloo B., van Druen E., Smit B., Slater R. (1999): Spec-
tral karyotyping: detection and identification of new chro-
Genet., 85, 5–181.

Beverloo M. (1987): The molecular genetics of the cancer. Sci-
ence, 235, 305–311.

Beverloo B., van Druen E., Smit B., Slater R. (1999): Spec-
tral karyotyping: detection and identification of new chro-
Genet., 85, 5–181.

Beverloo M. (1987): The molecular genetics of the cancer. Sci-
ence, 235, 305–311.

Beverloo B., van Druen E., Smit B., Slater R. (1999): Spec-
tral karyotyping: detection and identification of new chro-
Genet., 85, 5–181.

Beverloo B., van Druen E., Smit B., Slater R. (1999): Spec-
tral karyotyping: detection and identification of new chro-
Genet., 85, 5–181.

Beverloo B., van Druen E., Smit B., Slater R. (1999): Spec-
tral karyotyping: detection and identification of new chro-
Genet., 85, 5–181.

Beverloo B., van Druen E., Smit B., Slater R. (1999): Spec-
tral karyotyping: detection and identification of new chro-
Genet., 85, 5–181.


Figure 4:

A. Results of the fiber-FISH experiments with the following probe combinations and labels (in brackets): exp.1 probe mgf4(bio) - probe mgf18(dig); probe mgf18(bio) - probe mgf19(dig); probe mgf4(bio) - probe mgf19(dig). Digoxygenine labeled probes were detected with FITC (green), biotin labeled probes with Texas red (red), respectively.

B. Physical organization of the bovine STAT locus as revealed by fiber FISH analysis. Hybridization probes are depicted by lines. The whole STAT locus as analyzed by 3 different clones covers about 85 kb of genomic DNA.

Figure 5:

Localisation of ZuBeCa4 microsatellite on the blue fox chromosomes: (a) QFQ banding, (b) FISH; chromosome pair No 4 is indicated by arrows
Figure 1:  
a) Simmental bull  
c) Black Holstein Cow  
b) Red Holstein Cow  
d) Brown Swiss Cow  

Figure 2: Eringer cow "Queen" (Melitta, 1997)  

Figure 3: Clitoris protuberance