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TITLE: Chromosomal Abnormalities in the Development of Malignancies

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Hello, my name is Elena Repnikova. I am an Assistant Director of Cytogenetics and Molecular Genetics Laboratories at Children's Mercy Hospital and an Assistant Professor of Pathology and Pediatrics at University of Missouri in Kansas City. Welcome to this Pearl of Laboratory Medicine on "Chromosomal Abnormalities in the Development of Malignancies."

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Most structural chromosomal abnormalities appear during DNA replication or repair, when DNA is particularly sensitive to breakage and fusion. These events may occur in the result of recombination between repetitive or homologous DNA sequences, which can result in formation of variant translocations with or without DNA loss.

Structural abnormalities are classified as translocations, deletions, inversions, or duplications. Reciprocal translocations involve exchange of material between different chromosomes and are present in almost 50% of hematological malignancies.

Some chromosomal translocation acquired somatically may reactivate a proto-oncogene through mutation or increased expression. This could, in turn, disrupt the critical balance of cell proliferation, cell maturation, and cell death. In many cases, these chromosomal translocations fuse sequences of a transcription factor or tyrosine kinase receptor gene to unrelated genes, resulting in a chimeric protein with oncogenic properties.

Chromosomal abnormality appearing in one cell through activation of an oncogene or disruption of tumor-suppressor gene may allow the cell to proliferate rather than resulting in its death, and give rise to a clone with malignant potential. The clonal pattern of malignancies has been demonstrated with cytogenetic studies, molecular methods, and many other procedures.

As neoplastic cells proliferate, additional chromosomal abnormalities appear in sporadic malignant cells. These cells divide and contain the primary chromosome abnormality as well as acquire secondary abnormalities. Chromosome evolution is responsible for the complex karyotypes in many malignancies. Generally, the number of chromosome abnormalities and karyotype complexity in an abnormal clone correlates with tumor progression and may affect tumor response to therapy.

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Major analytic techniques that are used to detect chromosomal abnormalities include routine karyotype analysis, fluorescent in situ hybridization (FISH), and PCR-based reverse-transcriptase and quantitative reactions.

Routine karyotype analysis requires a specimen with live cells grown in culture. Treatment with various enzymes and stains results in unique chromosome banding pattern. Using chromosome size and banding pattern, a cytogeneticist can detect changes in chromosome number and major structural abnormalities. The limit of resolution for standard karyotype analysis is on the order of 5-10 Mb.

Fluorescent in situ hybridization, another technique allowing to detect chromosomal changes, provides an increased resolution and does not require dividing cells. The technique requires a physician to suspect the diagnosis for the proper choice of the correct probe for hybridization.

PCR-based clinical diagnostic tests such as reverse-transcriptase PCR and quantitative PCR are very sensitive and ideal for detection of point mutations and small insertions, deletions, translocations, and DNA fusion events. The major limitation of PCR-based techniques is the size of the amplified fragment which is usually less than 1 kb, which limits the number of translocation breakpoints that can be analyzed in a single PCR reaction.

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Karyotyping, FISH, and PCR-based assays enhance the knowledge of hematological neoplasms. Chronic myelogenous leukemia (CML) is one of them. It is a myeoloproliferative neoplasm that originates in an abnormal pluripotent bone marrow stem cell.

The disease can occur at any age but is most frequent in the 5th or 6th decades of life. CML is always associated with chromosome 9 and 22 translocation, which results in formation of chimeric transcript between the *ABL1* and *BCR* genes at 9q34 and 22q11.2, respectively. The derivative chromosome 22, also known as Philadelphia chromosome, is the first abnormality that has been associated with a specific malignant neoplasm. The *BCR-ABL1* chimeric gene leads to the production of abnormal tyrosine kinase. *BCR-ABL1* fusion gene can be detected by FISH with dual fusion dual color probe to *BCR* and *ABL1* revealing two fusion signals corresponding to the rearranged chromosomes 9 and 22, or RT-PCR technique. In the majority of patients with CML, an abnormal fusion protein p210 (210 kDa) with enhanced tyrosine kinase activity is found. Successful treatment of CML includes use of imatinib (Gleevac) to inhibit increased tyrosine kinase activity, thus interrupting its oncogenic signal.

There are three main clinical phases of CML: chronic, accelerated, and blast crisis. The chronic phase of CML is characterized by mild or no symptoms and less than 5% of blasts. At this stage, the only abnormality is the t(9;22). Accelerated and blast crisis stages are characterized in an increase in the number of blasts, worsening of clinical symptoms, and acquisition of additional chromosomal abnormalities.

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Acute myelogenous leukemia (AML) is defined by the presence of myeloblasts in the bone marrow, peripheral blood, and other tissues. Although AML more frequently affects adults in their 60s, it has been also described in children and young adults. Among the myeloid neoplasms, this leukemia

accounts for the majority of specific abnormalities and for large number of rearrangements, most of which are translocations. The AMLs with recurrent genetic abnormalities are characterized by the presence of well-established genetic abnormalities, the most common of which are t(8;21), inv(16) or t(16;16), t(15;17), and t(9;11). These chromosomal abnormalities have been associated with particular subtypes of AML, because only a particular subset of myeloid cells undergoes proliferation as a result of specific gene rearrangement.

AML with t(8;21) is one of the core binding factor myeloid leukemias that mainly affects adults. This translocation leads to *RUNX1-RUNX1T1* fusion and is generally associated with a favorable prognostic outcome.

AML with inv(16) or t(16;16) is characterized by the presence of myelomonocytic blasts and atypical eosinophils. The abnormality leads to fusion of *MYH11* and *CBFB*. The identification of this rearrangement by conventional cytogenetics might be challenging when chromosome morphology is poor. In those cases, FISH or RT-PCR can be helpful.

AML with t(15;17) leads to fusion between *PML* and *RARA* and is associated with a favorable prognosis and response to treatment with all-trans retinoic acid.

AML with t(9;11) leads to fusion of *MLLT3* and *KMT2A(8:57)* genes and is found in AML with monocytic or myelomonocytic phenotype. This is the most frequent translocation involving *KMT2A* gene. Large-scale studies have shown that this particular translocation is associated with intermediate prognosis.

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Acute lymphoid neoplasms include immature and mature neoplasms of B-cell and T-cells. Neoplasms of B-cell origin are more frequent than those of T-cell origin.

ALL is primarily a disease of children, with 75% of cases occurring in children under six years of age. B-cell ALL is a neoplasm of lymphoblasts committed to a B-cell lineage. Approximately 80% of cases are of a precursor B-cell phenotype. Major translocations in precursor B-cell ALL include:

- t(4;11) – which is the most common in children under 12 months and portends a poor prognosis. The *KMT2A* gene on chromosome 11 has many fusion partners. The most common partner gene is *AF4* on chromosome 4q21. Infants, especially those <6 months of age, have very poor prognosis.
- t(12;21) - 15 to 35% of pediatric B-lineage ALL: so far, the most frequent translocation in this group; rare or absent in adults and in infants, but common in children; with male and female being equally represented. This translocation results in the production of a fusion protein that is likely acting in a dominant negative fashion to interfere with normal function of the transcription factor RUNX1. t(12;21) has a very favorable prognosis and >90% of children with this translocation can be cured.
- t(9;22) – results in fusion of the *BCR* gene at 22q11.2 with *ABL1*, a tyrosine kinase. In ALL, this translocation is commonly seen in adult patients. In most childhood ALL cases, a variant fusion protein p190 kDA is seen. In both children and adults, t(9;22) ALL has poor prognosis among patients with ALL.

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Nearly 15% of children and 25% of adults diagnosed with ALL have T-cell ALL. This type of leukemia affects males more than females and generally affects older children more than does B-cell ALL. Patients usually have high white blood cell counts and may present with organomegaly, particularly mediastinal enlargement, and CNS involvement.

Most common chromosome abnormalities in T-cell ALL involve the 14q11 (*TCRA/D*) and 7q35(*TCRB*) regions. They juxtapose enhancer elements of the TCR genes with transcription factors involved in T-cell differentiation, thus deregulating hematopoiesis. Some of the common translocations are listed in the table.

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Mature B-cell neoplasms comprise about 90% of lymphoid neoplasms. Several mature B-cell neoplasms have characteristic genetic abnormalities that are important in determining their biological features and are useful in differential diagnoses. We will discuss some of these.

Follicular lymphoma is a neoplasm composed of follicle center (germinal center) B-cells, which usually has at least a partially follicular pattern. Follicular lymphoma accounts for about 20% of all lymphomas with highest incidence in the USA and Western Europe. It affects predominantly adults with a median age in the 6th decade. Follicular lymphoma is characterized by the translocation t(14;18)(q32;q21) and *BCL2* rearrangements.

Mantle cell lymphoma (MCL) is a B-cell neoplasm generally composed of monomorphic small to medium-sized lymphoid cells with irregular nuclear contours and a translocation involving cyclin D1. MCL comprises approximately 3-10% of non-Hodgkin lymphomas. It occurs in middle-aged to older individuals with a median age of about 60 years.

Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) is an extranodal lymphoma composed of morphologically heterogeneous small B-cells including marginal zone cells, cells resembling monocytoid cells, small lymphocytes, and centroblast-like cells.

Diffuse large B-cell lymphoma is characterized by diffuse proliferation of large neoplastic B-lymphoid cells with nuclear size equal or exceeding normal macrophage. Patients usually present with a rapidly enlarging tumor mass at single or multiple nodal or extranodal sites. In general, this lymphoma affects individuals of older age. Diffuse large B-cell lymphomas are very heterogeneous at the cellular level and cytogenetically diverse. Common recurrent abnormalities include t(14;18), which involves *IGH* and *BCL2*, the same translocation observed in follicular lymphoma. Translocations involving *BCL6* with more than 30 different partner genes translocated with this locus are detected in approximately 35% of patients with DLBCL. A *MYC* rearrangement with *IGH* break partner is observed in 10% of cases.

Burkitt lymphoma is a particularly aggressive lymphoma, often presenting at external sites. It is relatively common in children and accounts for approximately 35-50% of all childhood lymphomas. Burkitt lymphoma cells show clonal rearrangements affecting *MYC* at 8q24.2. The most common of these, t(8;14) with rearrangement of immunoglobulin heavy chain, is detected in about 75% of patients. Other two variant translocations with *IGK* or *IGL* light chains are seen in approximately 5-10% of patients. All these translocations lead to overexpression of *MYC.*

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Mature T-cell neoplasms are derived from post-thymic T cells. Mature T-cell lymphomas are uncommon lymphoid malignancies and are reported to constitute about 15% of non-Hodgkin lymphomas. The most common subtypes of mature T-lymphomas are prolymphocytic lymphomas and anaplastic large cell lymphomas.

T-cell prolymphocytic leukemia is an aggressive T-cell leukemia that affects approximately 2% of adults. The most common sites of involvement include peripheral blood, bone marrow, lymph node, spleen, and liver. The most common chromosome abnormalities involve T-cell receptor genes and T-cell leukemia 1 gene at 14q32.1

Anaplastic large cell lymphoma accounts for approximately 3% of all lymphomas. The majority of cases stain positive for the protein anaplastic lymphoma kinase (ALK). This lymphoma includes two main subtypes: ALK+ and ALK-. Cytogenetically, ALK+ ALCL is characterized by translocation involving *ALK* gene. The most common ALK rearrangement is t(2;5) leading to activation of ALK.

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Solid tumors are neoplasms arising from non-hematopoietic tissues of the body, which may be either benign or malignant, although most solid tumors for which molecular diagnosis is applied are malignant. Malignant solid tumors are divided into two broad categories, sarcoma and carcinoma, based upon their tissue of origin.

The chromosomal aberrations in solid tumors result in translocation, deletion, or amplification of target genes. Translocations are particularly frequent in sarcomas, where they usually create fusions of genes at the breakpoints of the participant chromosomes. Deletions are frequent in carcinomas, where they likely result in loss of tumor suppressor genes.

The most common genetic methods for analysis of chromosomal abnormalities in solid tumors include:

- conventional cytogenetics, which despite providing a genome-wide screen requires fresh tissue and more time and effort to set up culture specimen and analyze
- fluorescence in situ hybridization, a technique suitable for fresh, touch prep, smear, and paraffin section; however, it requires an ordering physician to suspect the diagnosis for the choice of the correct probe
- reverse transcriptase PCR is suitable for analysis on fresh, frozen, or paraffin-embedded tissues sensitive to detect a low level disease, but this technique is limited for use to molecularly known abnormalities.

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Soft tissue tumors represent a heterogeneous and complex group of mesenchymal lesions that may show a broad range of differentiation. Histologic classification is based upon morphologic demonstration of a specific line of differentiation. Soft tissue sarcomas compared with carcinomas and other neoplasms, constitute fewer than 1% of all cancers. Molecular cytogenetic analysis of tumor-specific chromosomal translocations and associated fusion gene transcripts offers a useful adjunct to the diagnosis of soft-tissue tumors.

Small round cell tumors are characterized by small, round, relatively undifferentiated cells. These include but are not limited to Ewing's sarcoma, desmoplastic round cell tumor, alveolar, and embryonal rhabdomyosarcoma.

Ewing's sarcoma is a highly aggressive bone and soft tissue tumor. Most Ewing's sarcomas contain chromosomal translocations involving the Ewing's sarcoma gene (*EWS*) on chromosome 22. The most common rearrangement is t(11;22) which results in oncogenic fusion of the *FLI1* gene, which encodes for a transcription factor with *EWS* gene. The Ewing's gene translocations can be detected cytogenetically by FISH or RT-PCR, which has a very high sensitivity for identification of breakpoint locations within the translocated genes.

Desmoplastic small round cell tumor usually arises from intra-abdominal soft tissues. Almost all cases include translocation 11;22 between *WT1* and *EWSR1* genes forming an oncoprotein which upregulates the expression of platelet-derived-growth factor-alpha (PDGFA).

Rhabdomyosarcoma is a tumor of skeletal muscle differentiation. The most common subtypes of rhabdomyosarcoma include embryonal and alveolar forms. The alveolar rhadbomyosarcoma is characterized by reciprocal translocations involving the *FOXO1* transcription factor on chromosome 13 (t(1;13) and t(2;13)) and *PAX7* and *PAX3* genes, respectively.

Congenital fibrosarcoma is a rare soft tissue tumor composed of malignant fibroblasts in a collagen background. It is highly cellular and composed of spindle cells arranged in fascicles. It is characterized by a diagnostic chromosomal translocation t(12;15), which is cryptic and may not be visible by traditional cytogenetic methods.

Clear cell sarcoma resembles malignant melanoma; however, histologically they are different. Clear cell sarcomas generally present as isolated masses in deep soft tissues. Most of clear cell sarcomas contain a chromosomal rearrangement t(12;22) which serves as reliable marker to distinguish these tumor types. This translocation fuses the *ATF1* gene on chromosome 12 and *EWSR1* gene on 22, which produces an activated form of a transcription factor.

The most diagnostically useful aberration in malignant adipose tumors is a translocation between chromosomes 12 and 16. The translocation is found in myxoid liposarcomas and results in fusion of the *DDIT3* gene on chromosome 12 with the *FUS* gene on chromosome 16.

A specific chromosomal abnormality, t(9;22)(q22;q12), characterizes this extraskeletal myxoid chondrosarcoma, though variant translocations have been also described. Variant translocations have been also reported: t(9;17)(q22;q11) and t(9;15)(q22;q21). All three translocations result in fusion of the *NR4A3* gene at 9q22 with the *EWSR1* gene at 22q12, or with *TAF15* gene at 17q11, or with *TCF12* gene at 15q21.

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Renal cell carcinoma is the most common tumor arising from the kidney. Prognosis is highly dependent on histological subtype and tumor stage at diagnosis. The most common histological subtypes include clear cell renal cell carcinoma, papillary renal cell carcinoma, chromophobe, renal cell carcinoma with Xp11.2 or 6p21 translocations, oncocytoma, Wilms tumor, mesoblastic nephroma, and others. Different subtypes are characterized by different chromosomal abnormalities.

For example, chromosome 3p deletion is the most frequent cytogenetic aberration in clear cell renal cell carcinoma, which results in loss of tumor suppressor gene, *VHL*. Common cytogenetic abnormalities in papillary renal cell carcinoma include gains of chromosomes 7, 17 and loss of chromosome Y. Most chromophobe carcinomas have hypodiploid karytotype including monosomies of chromosomes 1, 2, 6, 10, 13, 17, and 21.

Pediatric renal cell carcinomas are uncommon, and often have translocations involving the X chromosome and chromosome 6. The most common translocation is t(X;1) resulting in fusion of *PRCC* and *TFE3* gene on chromosome X.

Oncocytoma in most cases has a loss of entire chromosome 1, or del 1p, and loss of a sex chromosome (X or Y).

Wilms' tumors are the most common type of renal cancer in children and typically include trisomies of chromosome 6, 8, 12, 18, and deletions of 11p and 16q. The most common rearrangements are isochromosome 1q and 7q.

Mesoblastic nephroma is the common renal tumor found in neonates. Most common translocation is t(12;15) which results in oncogenic fusion of the *ETV6* and *NTRK3* genes, which can be easily detected by FISH.

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Deletions rather than translocations are common in nonmesenchymal tumors, where they likely result in loss of tumor suppressor genes. Examples of characteristic chromosomal abnormalities for some of them are presented in the table.

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Points to remember about chromosomal abnormalities in the development of malignancies:

- Recurrent translocations in neoplasms improve understanding of genetic mechanisms and pathways involved in leukemogenesis
- In leukemias and solid tumors, the presence of specific translocation becomes a marker for disease monitoring and helps identifying patients
- Recurrent chromosomal translocations help to clone the genes and find molecular targets for treatment options
- The most commonly used analytic methods for detection of chromosomal abnormalities include chromosomal analysis, FISH, and rt-PCR

Slide 15: References

Slide 16: Disclosures

Slide 17: Thank You from www.TraineeCouncil.org
Thank you for joining me on this Pearl of Laboratory Medicine on "Chromosomal abnormalities in the development of hematological malignancies." My name is Elena Repnikova.