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Genetic Technologies in Cancer Investigation – Applications in Aggresive Lymphoid Malignancies

Nicoleta Mariana BERBEC, MD, PhD[a,b*]; Aurora ARGHIR, MD, PhD[c*]; Anca VITCU, PhD[d]; Silvana ANGELESCU, MD, PhD[a,b]; Andrei COLITA AMD, PhD[a,b]; Anca CIOBANU, MD[b]; Sorina Mihaela PAPUC, Eng[c]; Andreea-Cristina TUTLAN-CUNITA, PhD[c]; Anca Roxana LUPU, MD, PhD[a,b]

[a]"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
[b]Hematology Department, Coltea Clinical Hospital, Bucharest, Romania
[c]"Victor Babes" National Institute of Pathology, Bucharest, Romania
[d]"Alexandru Ioan Cuza" University of Iasi, Romania
*These authors equally contributed to this paper

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BACKGROUND

Cancer is, at cellular level, a genetic disease; genetic anomalies alter the balance between cell proliferation, survival, and differentiation. A vast array of chromosomal abnormalities have been found in virtually all tumor types; currently, it is accepted that chromosomal anomalies are consistent features of cancer, and, in most cases, pathogenetically significant events. However, only a fraction of the cancer-associated genetic aberrations have been identified. The continuous progress of genomic technologies, leading to increased resolution and detection of genetic events such as copy-neutral loss of heterozygosity, is expected to lead to the identification of additional genetic anomalies that ultimately will improve the therapeutic strategies and clinical management of hemato-oncological patients.

There is a growing and general recognition of the significance of chromosomal changes in cancer initiation and progression. The World Health Organization Classification of Tumors uses these aberrations to define specific disease entities. Many genetic alterations proved to be powerful determinants of prognostic. By providing insights into the pathogenetic mechanisms of cancer, the characterization of chromosomal abnormalities paved the way to the identification of therapeutic targets that were successfully approached by molecularly oriented strategies. Therefore, genetic testing can be

Address for correspondence:
Silvana Angelescu, MD - Coltea Clinical Hospital, Hematology Department – 1, I.C. Bratianu Ave, 030171, Bucharest. Phone.: +40724278331
E-mail: nicole_olt@yahoo.com

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used to identify the patients that will benefit from a particular molecular targeted therapy.

Acute lymphoblastic leukemia (ALL) and aggressive non-Hodgkin lymphoma (NHL) represent a major cause of morbidity from hematologic neoplasms in adults. Acute leukemias have been subjected to intense investigations and are consequently among the best characterized disorders at genetic level. This is due to the easy access to malignant cells as well as to the successful implementation of cytogenetic techniques (1). However, recently performed high resolution, genome wide studies identified multiple novel genetic changes addressing critical cellular pathways. Among these, worth mentioning are alterations of genes coding for transcription factors involved in lymphoid development, as well as oncogenes and tumor suppressor genes. For NHL there is far less understanding of the pathogenetic mechanisms and a high degree of heterogeneity between groups and subgroups.

Although genomic and genetic studies provided insights into tumorigenesis and led to the identification of biological and clinical subgroups, future studies are needed to unravel the genetic/genomic architecture and to correlate it with clinical outcome.

The genome-wide approaches proved to be powerful tools in detecting genetic lesions in human lymphoid malignancies suggesting that ongoing genomic analyses of larger patients cohorts will continue to bring novel insights into leukemogenesis; thus an increased understanding and ultimately a better patient care will be achieved.

Cancer stands as a highly complex and heterogeneous set of diseases, in which genetic lesions play a leading role. Genomic, epigenomic, transcriptomic and proteomic alterations that lead to gain of function of oncoproteins or loss of function of tumor suppressor proteins underlie the development of virtually all cancers. Nevertheless, the mechanisms of malignant transformation are only partially understood (2).

Clonal chromosomal aberrations detected in over 60,000 cases are centralized in Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer (3). Major research efforts jointed in order to decipher the complexity of cancer genome, leading to consortia committed to exploitation of high-throughput genome-wide technologies and to dissemination of knowledge towards the research and medical community (Cancer Genome Characterization Initiative, Cancer Genome Anatomy Project, Cancer Genome Project).

Both chromosomal rearrangements and genomic imbalances (gains and losses) have been detected in malignant cells, usually as acquired alterations. The functional consequences of balanced rearrangements are either the formation of fusion chimeric genes either the deregulation of expression of normal genes. Fusion genes can be localized on episomes (e.g. ABL1/NUP214) and thus can only be detected by means of molecular cytogenetic/genetic methods (4). Genomic imbalances most likely act through gene dosage alterations (increased or reduced); genomic losses of non-coding genes proved significant for pathogenesis as these genes codes for small RNA molecules involved in post-transcriptional gene expression regulation (5). Recent studies also revealed that loss of heterozygosity (LOH) and copy-neutral LOH (CN-LOH) (6) occurs frequently in cancer. These findings suggest that the genomic regions involved in acquired uniparental disomies contain genes that are critical for neoplastic transformation and/or progression. There is a vast amount of evidence in the literature that detection of CN-LOH in cancer is clinically relevant (7-12). ❑

CHROMOSOMAL ANOMALIES IN ACUTE LYMPHOBLASTIC LEUKEMIA

Acute lymphoblastic leukemia (ALL) represents a heterogeneous group of disorders characterized by uncontrolled clonal proliferation of lymphoid precursor B and T cells. ALL and lymphoblastic lymphoma are now considered the same disease based on morphologic, genetic and immunophenotypic features; currently these two disorders are unified as precursor B-cell and T-cell lymphoblastic leukemia/lymphoma within the 2008 World Health Organization (WHO) classification (13).

Chromosome abnormalities in ALL are being detected with a variable incidence depending on the techniques used and are strong prognostic determinants. Chromosome abnormalities in ALL can be numerical, structural or both.

The genetic abnormalities identified by metaphase cytogenetics/FISH/RT-PCR in B-ALL are predominantly balanced rearrangements

leading to chimeric fusion genes [e.g. BCR-ABL1- t(9;22)(q34;q11); MLL-AF4 - t(4;11)(q21;q23); TEL-AML1 - t(12;21)(p13;q22); MLL-v - t(11;v)(q23;v)] or deregulation of expression of a normal gene [e.g. c-MYC-IgH - t(8;14)(q24;q32) and IL3-IgH -t(5;14)(q31;q32)]. Translocation t(9;22) has a frequency of 25-30% in adult ALL (40% of the patients over 50 years old) and 5% in children, being the most frequent structural chromosome anomaly in adult ALL. T-ALL is characterized, in most patients investigated by cytogenetic approaches, by one of the following abnormalities: TAL1 deletion; t(8;14)(q24;q11); t(10;11)(q24;q11); t(11;14)(p15;q11); t(11;14)(p13;q11); t(1;14)(p32;q11); inv(14)(q11q32).

With the advent of high resolution, genome-wide approaches novel genetic lesions are constantly unraveled in ALL Several microarray studies indicated multiple regions of recurrent genomic aberrations, previously undetected by classical approaches (14,15). These studies revealed that there are numerous submicroscopic aberrations that alter critical cellular pathways and have pathogenetic significance as well as prognostic relevance. The genes affected by focal genomic deletions code for lymphoid transcription factors, tumor suppressors, regulators of apoptosis etc. Similar to other malignant hemopathies, focal genomic gains are less frequently identified in lymphoproliferative disorders. ❑

MOLECULAR ARCHITECTURE OF MALIGNANT LYMPHOMAS

Malignant non-Hodgkin lymphomas comprise a heterogeneous group of tumors having as cells of origin lymphocytes at different maturation stages. Various chromosomal and molecular rearrangements as well as genomic imbalances were described in NHL; cytogenetic findings play an important pathogenetic role and are correlated with the histological and imunofenotypic features. However, genetic alterations are not used to define distinct entities within 2008 WHO classification of mature cell neoplasms. This reflects the intrinsec heterogeneity as well as the incomplete understanding of NHL. Among NHL, diffuse large B-cell lymphoma (DLBCL) and peripheral T-cell lymphoma (PTCL) have an aggressive behavior and a biological and clinical heterogenity that lead to different treatment outcomes. DLBCL is one of the two most common types of NHL. The molecular events that underlye DLBCL and PTCL are poorly understood. Traditionally, diagnostic of NHL is based on histopathological procedures supplemented with immunophenotyping, cytogenetics, molecular genetic analyses and clinical information. Cytogenetic studies have proved to be difficult in comparison with acute leukemia. The development of FISH techniques enabled chromosomal studies in NHL (touch preparation). Modern diagnoses have to be supplemented with molecular diagnostics in order to improve the medical care and to open new therapeutic strategies for patients. Genome wide approaches in NHL focused mainly on gene expression profiling. Thus, there are relatively few reported studies investigating the role of CN-LOH at genome-wide level, in NHL. ❑

GENETIC TECHNOLOGIES DEVELOPMENT

Cytogenetic findings provide valuable diagnosis and prognostic information, as well as a better understanding of disease biology and underlying pathogenetic mechanisms. Chromosomal studies (metaphase cytogenetics and FISH) offered the first glimpse into the pathogenesis of cancer, mainly in hematological malignancies.

Metaphase cytogenetics and mutational analysis of single/several genes were until recently the major contributors to the body of data regarding the genetic lesions that contribute to the pathogenesis of lymphoid leukemia/lymphoma. These approaches have nevertheless been insufficient for accurate describing the complex architecture of leukemia genome. Moreover, neither FISH nor conventional metaphase cytogenetic can identify copy-neutral loss of heterozygosity (CN-LOH), thus genome-wide approaches (e.g. Single Nucleotide Polymorphism – SNPs array) are powerful tools for cancer genome investigation (15). The introduction of microarray technologies in medical practice requires a thorough integration of existing standard methods with newly developed ones. Several consortia act towards standardization of microarrays for clinical diagnosis. Among these, Cancer Cytogenomics Microarray Consortium (CCMC) aims to develop cancer-specific DNA microarray standards for research and clinical applications and to conduct multi-institutional clinical trials. ❑

BIOINFORMATICS TOOLS

Modern high throughput technologies require a complex armamentarium of bioinformatics tools. Bridging biomedical approaches, bioinformatics analysis and mathematical tools have increased the investigative power of modern technologies. Genome wide high resolution studies generate vast amount of data that have to be thoroughly analyzed. Regarding the methodology on data analysis, recent developments were reported in Bayesian bioinformatics relevant to biomedical systems. Such methods can be used to discover sparse latent structure, while accounting for uncertainty. Given the enormous dimensional model, spaces that are routinely encountered in bioinformatics applications, it is crucial to account for uncertainty in model selection in conducting inferences and predictions.

Bayesian methods are valuable whenever there is a need to extract information from data that are uncertain or subject to any kind of error or noise (ex. measurement error and experimental error, as well as noise or random variation intrinsic to the process of interest). Bayesian methods offer a number of advantages over more conventional statistical techniques that make them particularly appropriate for complex data.

Algorithms and methodologies originating from mathematics and computer science have been adapted and extended with respect to topology, motifs, modules and network comparison. The extracted information and learned knowledge are applied on specific biological/medical problems of interest, such as the identification of prognostic biomarkers of cancer to facilitate the understanding of the underlying mechanisms of biological systems and benefit the health care.

It has become increasingly obvious that a comprehensive approach of mining is necessary to extract meaningful information from extensive data sources. Systems biology was introduced as a method of lateral thinking. It aims to provide a structured framework to mine high-throughput data by using an understanding of biological processes as integrated systems. In this approach, all parts of the biological system are studied simultaneously, taking into consideration their dynamic interactions within temporal, spatial, and physiological contexts. It is important to stress that systems biology approaches not only focus on the molecular determinants but they also take into account their interactive relationships within the system. To achieve this goal, functional models are constructed using experimental data from as many diverse sources as possible. The identified functional modules are then linked and compared to available models in public repositories. Mathematical and computational analyses are then used to generate new functional models based on the initial set of data and the available information within the public databases. These combined models are then refined through many iterative cycles of computational simulation, prediction, and validation with other experimental results. This integrative approach is essential to understand the complexity of common human diseases like cancer and to generate new hypothesis, starting from a hypothesis-independent approach such as DNA microarray. Once a model has been constructed, further study and experimentation is needed to more persuading demonstrate understanding of the state of a given molecular network, the interactions, and, finally, how the networks change in response to different genetic and environmental contexts. ❑

CONCLUSIONS

Reviewing all of the above, there are several conclusions to be drawn:

1. presently, there is a paucity of SNPs array studies in NHL;
2. from the medical care point of view, the establishment of a set of biomarkers to be detected in laboratory tests would be an advantage, beside histological and immunophenotypic criteria used in the diagnosis of lymphoid malignancies;
3. due to the heterogeneity of lymphoid malignancies, there is a pressing need for collaborative studies and multidisciplinary approaches;
4. there is also a heterogeneity of experimental approaches and investigative methodologies, thus the need to standardize the algorithms used. ❑

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References

1. **Rowley JD –** Chromosomal translocations: revisited yet again. *Blood* 2008; 112:2183-2189
2. **Hanahan D, Weinberg RA –** The hallmarks of cancer. *Cell* 2000;100:57-70
3. Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer (2011). Mitelman F, Johansson B and Mertens F (Eds.), http://cgap.nci.nih.gov/Chromosomes/Mitelman
4. **Graux C, Cools J, Melotte C, et al. –** Fusion of NUP214 to ABL1 on amplified episomes in T-cell acute lymphoblastic leukemia. *Nat Genet* 2004; 36:1084-1089
5. **Calin GA, Sevignani C, Dumitru CD, et al. –** Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proc Natl Acad Sci USA* 2004; 101:2999-3004
6. **Tuna M, Knuutila S, Mills GB –** Uniparental disomy in cancer. *Trends Mol Med.* 2009; 15:120-128
7. **Kawamata N, Ogawa S, Zimmermann M, et al. –** Molecular allelokaryotyping of pediatric acute lymphoblastic leukemia by high resolution single nucleotide polymorphism genomic microarray. *Blood* 2008; 111:776-784
8. **Paulsson K, Cazier JP, MacDougall F, et al. –** Microdeletions are a general feature of adult and adolescent acute lymphoblastic leukemia: Unexpected similarities with pediatric disease. *Proc Natl Acad Sci USA* 2008; 105:6708-6713
9. **Gupta M, Young BD –** Application of SNP genotype arrays to determine somatic changes in cancer. *Methods Mol Biol.* 2009; 538:179-206
10. **Cheung KJ, Delaney A, Ben-Neriah S, et al. –** High resolution analysis of follicular lymphoma genomes reveals somatic recurrent sites of copy-neutral loss of heterozygosity and copy number alterations that target single genes. *Genes Chromosomes Cancer.* 2010; 49:669-81
11. **Paulsson K, Forestier E, Lilljebjörn H, et al. –** Genetic landscape of high hyperdiploid childhood acute lymphoblastic leukemia. *Proc Natl Acad Sci USA* 2010; 107:21719-21724
12. **de Leeuw N, Hehir-Kwa JY, Simons A, et al. –** SNP array analysis in constitutional and cancer genome diagnostics-copy number variants, genotyping and quality control. *Cytogenet Genome Res.* 2011; 135:212-21
13. **Vardiman JW, Thiele J, Arber DA, et al. –** The 2008 revision of the WHO classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood* 2009; 114:947-951
14. **Mullighan CG, Miller CB, Phillips LA, et al. –** BCR-ABL1 lymphoblastic leukemia is characterized by the deletion of IKAROS. *Nature* 2008; 453:110-114
15. **Mullighan CG, Downing JR –** Genome-wide profiling of genetic alterations in acute lymphoblastic leukemia: recent insights and future directions. *Leukemia* 2009; 23:1209-1218.