GENOMIC PROFILE OF ANTHRACYCLINE RESISTANCE IN CHILDHOOD ACUTE LEUKEMIA

Acute leukemias (AL), including acute lymphoblastic leukemia (ALL) and acute myeloblastic leukemia (AML), are the most common childhood cancers. ALL accounts for 80% of cases under the age of 18, while AML affects about 15% of children with AL. Chemotherapeutic protocols use anthracycline drugs (doxorubicin, daunorubicin, idarubicin, mitoxantrone). One of the most serious problems of leukemia treatment is the phenomenon of cytostat resistance. Microarray technology makes it possible to gain a detailed understanding of the mechanisms responsible for differential drug response at multiple levels, including the genome and transcriptome.

The aim of this study was to identify potential determinants of insensitivity to selected anthracyclines in the context of studies using microarray (CGH and expression) techniques.

The study included pediatric patients diagnosed with ALL or AML from whom marrow was harvested. Material for the study was isolated from mononuclear cells, using columnar methods. DNA was needed for aCGH experiments, while RNA was needed for expression arrays. Samples were analyzed qualitatively and quantitatively, labeled, subjected to fragmentation reactions and purified. Preparations with the highest quality were hybridized to the corresponding arrays. All steps of laboratory experiments were carried out according to the protocols of reagent manufacturers. The results, obtained in microarray experiments with fluorescent probes, were subjected to detailed bioinformatics and statistical analysis. The results from microarray analysis were verified by qPCR for a selected group of genes. Characteristic cytogenetic rearrangements and genes with differential expression levels were identified. An ontological analysis was also carried out.

Based on the results of the analyses, it was indicated that the basis of anthracycline resistance in pediatric acute leukemia is a complex phenomenon. Underlying it are not only deletions or duplications of chromosome fragments, but also more subtle changes in the expression levels of a number of diverse genes. Genes responsible for nucleic acid metabolism, including transcription factors, were the most abundantly represented. In addition, genes regulating apoptosis, integrin and chemokine signaling pathways, as well as genes involved in the activation of B and T lymphocytes were also significant. The aforementioned perturbations of intracellular mechanisms mainly lead to bypassed apoptosis of leukemic cells, their increased proliferation, disruption of intercellular adhesion, as well as inhibition of B and T lymphocyte activation.

The cytogenetic and expression profiles analyzed enabled the identification of new markers of anthracycline insensitivity. Based on the results, it is possible to identify new targets for further scientific research, which can support the effectiveness of anti-leukemia therapy. Among the identified new potential markers are B and T lymphocyte receptors (*CD* genes), genes responsible for receptor binding (*S100* genes) and cellular transport (*SLC* genes), cell signaling pathway (*DUSP* genes), integrin (*ITGB2*) and caspase (*CASP* genes) signaling pathways. Chromosomal rearrangements, i.e. del5q35.3-q32, amp8p11.21-p12 and del9p21.3, also appear to be important in breaking the phenomenon of drug resistance to anthracyclines.

The compilation of transcriptomic and genomic data obtained using advanced molecular techniques, provides a multidimensional view of the problem of drug resistance. Based on the results, exposure of leukemic blasts to anthracyclines initiates a complex cellular response that reflects global changes in the expression of the biologically relevant genes described above.

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