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# ON THE ALL-UKRAINIAN SCIENTIFIC-PRACTICAL CONFERENCE WITH INTERNATIONAL PARTICIPANTS "MOLECULAR BASES AND CLINICAL PROBLEMS OF DRUG RESISTANCE" HELD ON NOVEMBER 2006 IN R.E. KAVETSKY INSTITITE OF EXPERIMENTAL PATHOLOGY, ONCOLOGY AND RADIOBIOLOGY, NAS OF UKRAINE, KYIV, UKRAINE

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## DRUG RESISTANCE: THE KEY PROBLEM OF CHEMOTHERAPY AND THE PERSPECTIVE WAYS TO OVERCOME IT

***V.F. Chekhun***

*R.E. Kavetsky Institite of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine, Kyiv, Ukraine, E-mail: chekhun@onconet.kiev.ua*

The history of chemotherapy (CT) of cancer patients is relatively short, but dramatic. "The magic bullet", that was a dream of P. Ehrlich, quickly changed its trajectory and acquired the effect of boomerang. This statement is supported by the fact of just 10 years period between the first application of CT and an appearance of the first reports on drug resistance at the beginning of 60-s. At the beginning of the era of intensive CT, natural resistance to medicinal preparations has been registered in 20% of patients. According to the statistical data of American Anticancer Society, in 80-s in 50% of primary registered patients with cancer natural drug resistance has been registered; after the therapy it has been detected in 49% of treated patients. That's why presently there is no necessity to stress the importance of the problem of resistance of tumor cells to medicinal preparations (MP). The certain positive efficacy of CT is achieved only due to the development and application of new cytostatics and their combined use. However, such approach only accelerates the formation of cross-resistance making drug resistance more severe and complexed problem. The rate of the development of this problem is higher than that of appearance of new cytostatics, thus complicating the selection of an adequate scheme of CT in each particular case.

The complexity of the problem of drug resistance manifests itself also in the fact that the characteristic markers are not unique; they are exhibiting themselves just in altered quantitative or qualitative relation of the functioning regulatory molecules. At the same time one should note that the frequency of genetic abnormalities in tumor cells is by 3 orders higher than in normal cells. The cell that survives under cytostatic therapy acquires additional structural-functional changes at different levels of its organization. So, optimal conditions for tumor progression and metastasis are created.

The results of own researches and analysis of the data of literature allow to separate tree interrelated levels of the formation of drug resistance: 1 — systemic level (pharmacokinetics, metabolism, neuro-humoral regulation); 2 — tissue level (extracellular matrix, vascularization system, tumor microenvironment); 3 — cellular level (membrane transport, pharmacogenetic, genetic, epigenetic and signal-apoptotic levels). Each of the mentioned levels possesses its own system of coordinates and is under intensive investigation. Significant progress in understanding the mechanisms of the formation of drug resistance has been made after deciphering of genome, when as a "bridge" between the function of gene and clinical signs of pathological process appears a new scientific discipline — proteomics. Due to the studies of proteins, a possibility to determine molecular profile of sensitive and resistant tumor cells has been achieved, and it plays an important role for determination of the course of the disease and efficacy of CT with the account of individual characteristics of the patient. For example, hyperexpression of P-gp evidences on the resistance to antracyclines, taxanes and other preparations of natural origin; activation of the enzymes of GST family and alteration of expression levels of CD-95 Fas-dependent ligands characterizes the resistance of tumor to cisplatin; amplification of gene coding dihydrofolate reductase and the decrease of activity of DNA topoisomerase-II reflect the resistance to methotrexate and etoposide. The obtained data create the grounds for the targeted therapy and show promising results *in vitro*.

Recently the attention of scientists is concentrated around epigenetic alterations in tumor cells. We hope that the correction of the processes of DNA methylation, modification of structural organization of chromatin and microRNA level are directed on overcoming drug resistance.

## ROLE OF MICRORNAOME CHANGES IN DRUG RESISTANCE OF BREAST CANCER CELLS

*O.V. Kovalchuk[1], I.P. Pogribny[2], V.F. Chekhun[3]*
*[1]Department of Biological Sciences, University of Lethbridge, AB, Canada T1K3 M4*
*[2]Division of Biochemical Toxicology, National Center for Toxicological Research, Jefferson, AR 72079*
*[3]Department of Mechanisms of Anticancer Therapy, R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, Kyiv Ukraine*

Many human cancers, including breast cancer, fail respond effectively to chemotherapy, and cancers that initially respond often acquire drug resistance. Presently, two hypotheses, genetic and epigenetic, have been proposed to explain mechanisms of acquired cancer drug resistance. "Genetic" is defined as a heritable change in the DNA sequence and suggests that occurrence of drug-induced random mutational events leads to formation of drug-resistant cells from sensitive cells. "Epigenetic" refers to the gene expression and chromatin changes and suggests that induction of epigenetic alterations results in resistance to cytotoxic drugs.

While the role of various genetic changes in breast cancer drug resistance has been extensively studied, the contributions of epigenetic alterations remained unexplored.

We have recently shown that epigenetic changes play crucial role in resistance of breast cancer cells to doxorubicin and cisplatinum. Another epigenetic mechanism of gene expression control via the function of small regulatory RNAs, particularly — microRNAs (miRNAs) remained unexplored. miRNAs are evolutionally conserved small single-stranded non-protein-coding RNA molecules presently recognized as major negative gene regulators. In mammals, miRNAs negatively regulate their targets by either binding to imperfect complementary sites within the 3′-untranslated regions of their mRNA-targets, or by targeting specific cleavage of homologous mRNAs. In the first case, miRNAs reduce protein levels of target genes by post-transcriptionally repressing target-gene expression without affecting mRNA levels of these genes, whereas in the second case, miRNAs induce the degradation of target mRNAs. In addition, recent findings suggest that miRNAs may act as regulators of various epigenetic processes. They play crucial role in regulating cellular differentiation, proliferation, and apoptosis. In light of these considerations, we hypothesized that microRNAome changes may play important role in the resistance of cancer cells to chemotherapeutic drugs.

miRNA microarrays were used to analyze the miRNA expression patterns in the human breast adenocarcinoma MCF-7 cell line and its drug-resistant to doxorubicin (DOX) MCF-7/DOX and to cis-Dichlorodiammine platinum (II) (cisDDP) MCF-7/cisDDP variants. Microarray data were confirmed using qRT-PCR. The cluster analysis revealed that MCF-7 breast cancer cells with acquired resistance to DOX and cisDDP are characterized by significant change in the miRNAome profiles. Importantly, in addition to the distinctive microRNAome signatures of MCF-7/DOX and MCF-7/cisDDP cells, we identified 35 down-regulated and 25 up-regulated miRNAs with simiar expression patterns in both MCF-7/DOX and MCF-7/cisDDP resistant cells at a level of $p < 0.01$. These results indicate that development of resistance to chemotherapeutic agents with different mode of action is associated with alteration of some common pathways, and this may to cancer drug cross-resistance development. Cluster of down-regulated miRNAs in drug-resistant cells consisted of, for instance, *let 7a, let 7f, let 7d, mir-34a, mir-15b* and *mir-149* controlling expression of *MRP1, MDR1, BRCA1,* and *BRCA2* transporter-genes; *mir-27b* regulating *CYP3A*. In contrast, number of microRNAs, such as *mir-214, mir -28, let-7i*, cluster *mir-17-92* regulating apoptosis-related genes, was up-regulated in resistant cells. These results indicate the importance of microRNAome deregulation in development of cancer drug resistance. The potential role of differentially expressed miRNAs in the generation and maintenance of the drug resistance phenotypes will be discussed.

## EPIGENETIC ASPECTS OF ACQUIRED CANCER CELL CHEMORESISTANCE

***[1]I.P. Pogribny, [2]O. Kovalchuk, [3]V.F. Chekhun***
*[1]National Center for Toxicological Research, Jefferson, AR, USA, igor.pogribny@fda.hhs.gov*
*[2]University of Lethbridge, Lethbridge, AB, Canada, olga.kovalchuk@uleth.ca*
*[3]R.E. Kavetsky Institite of Experimental Pathology, Oncology and Radiobiology, Kyiv, Ukraine, E-mail: chekhun@onconet.kiev.ua*

The successful treatment of cancer requires a clear understanding of multiple interacting factors involved in the development of drug-resistance. Presently, two hypotheses, genetic and epigenetic, have been proposed to explain mechanisms of acquired cancer drug resistance. The absence of convincing evidence that genetic changes have a role in acquired clinical resistance following anticancer therapy undermines the genetic hypothesis. In contrast, a number of studies indicate a substantial involvement of epigenetic mechanisms in drug-resistant cancer cells, including changes in DNA methylation and histone modification patterns.

In the present study, we examined the alteration in epigenetic mechanism in drug-resistant MCF-7 human breast cancer cells induced by doxorubicin (DOX) and cisplatin (cisDDP), two chemotherapeutic drugs with different modes of action. The MCF-7 cells with acquired drug resistance to DOX are characterized by increased expression of P-glycoprotein (Pgp), whereas MCF-7 cells resistant to cisDDP are characterized by increased expression of GST$\pi$. Despite of that, both of these drug-resistant cell lines displayed similar pronounced changes in global epigenetic landscape showing loss of global DNA methylation, loss of histone H4K20 trimethylation, and diminished expression of Suv4-20h2 histone methyltransferase

compared to parental MCF-7 cells. In addition to global epigenetic changes, MCF-7/DOX and MCF-7/cisDDP drug-resistant cells are characterized by extensive alterations in region-specific DNA methylation, which was evidenced by the appearance of number of differentially methylated DNA genes. The detailed analysis of hypo- and hypermethylated DNA sequences revealed that acquisition of drug-resistant phenotype of MCF-7 cells to DOX and cisDDP, in addition to specific alteration induced by particular drug only, is characterized by three major common mechanisms: dysfunction of genes involved in estrogen metabolism (sulfatase 2 and estrogen receptor α), apoptosis (p73, α-tubulin, BCL2-antagonist of cell death, tissue transglutaminase 2 and forkhead box protein K1), and cell-cell contacts (leptin, stromal cell derived factor receptor 1, activin A receptor E-cadherin). Furthermore, the results showed that two contradictory hypo- and hypermethylation processes enhance and compliment each other in the disruption of these pathways.

Recently emerged evidence suggests involvement of miRNAs in oncogenesis; however, their contribution to drug resistance development is currently unknown. Comparison of miRNA expression profiles between MCF-7/DOX and MCF-7/cisDDP and MCF-7 cells showed substantial changes in level of miRNAs. MCF-7/DOX and MCF-7/cisDDP cells displayed 48 and 57 down-regulated and 36 and 37 up-regulated miRNAs compared to MCF-7 cells, respectively. Interestingly, most of these differentially expressed miRNAs were common to both resistant cells. Additionally, we identified a number of common up-regulated miRNAs involved in regulation of apoptosis.

These results provided evidence that epigenetic changes are an important feature of cancer cells with acquired drug-resistant phenotype and may be a crucial contributing factor to its development. Furthermore, deregulation of similar pathways may explain the existence and provide mechanism of cross-resistance of cancer cells to different types of chemotherapeutic agents.

## ANTI-CANCER DRUG RESISTANCE IN SOME PROSTATE CANCER CELL LINES MAY BE EXPLAINED BY IMPAIRED PTEN PHOSPHATASE — FABP4 PROTEIN SIGNAL TRANSDUCTION BRANCH

*O.M. Gorbenko[1], D.D. Volkova[1], V.V. Filonenko[1], G. Panayotou[2], O.M. Zhyvoloup[1], I.T. Gout[1, 3]*

*[1]Department of Cell Signal Systems, Institute of Molecular Biology and Genetics, NAS of Ukraine, Kyiv, Ukraine*
*[2]B.S.R.C. "Alexander Fleming", Vari, Greece,*
*[3]Royal Free and University College Medical School, University College London, London, United Kingdom*
*Corresponding author: Olena Gorbenko, o.m.gorbenko@imbg.org.ua*

***Aim of investigation.*** Identification of novel functional links between tumor-suppressor PTEN and proteins, involved into signal transduction pathway during tumorigenesis.

***Object and methods.*** PTEN, which acts as lipid and protein phosphatase, is a key inhibitor of PI3K/Akt pathway. PTEN mutations or deletions are shown to be one of the possible causes of anti-cancer drug resistance in wide range of human cancers, including glioblastoma, melanoma, prostate, and breast tumours, due to constitutive Akt activity. It was also shown that PTEN could be a potential target in anti-obesity and diabetes mellitus treatment. Recently, it was revealed that thiazolidinediones, especially Rosiglitasone and Lovastatin, commonly used as anti-diabetes cure, could inhibit cell growth and proliferation by inducing PTEN expression through PPARgamma activation. On the other hand, the studies of ectopical PTEN overexpression in prostate cancer cell lines reveal, that increased PTEN level leads to the enhanced chemosensitivity and apoptosis in PTEN-null PC-3, LNCaP and LNCaP-Rf cells, however, not in DU145 cells containing functional PTEN. This may be explained by lacking the functional target located down-stream of PTEN signaling.

To clarify this assumption DuplexA™ yeast two-hybrid system followed by BIAcore, GST-pull down assay and gel-filtration assay have been applied.

***Results.*** Yeast two-hybrid screening of Mouse Embryo cDNA library allowed us to identify a novel PTEN-binding protein — FABP4, which is a marker of terminal stage of adipocyte differentiation and its expression is also controlled by PPARgamma. PTEN/FABP4 complex formation has been confirmed *in vivo* by yeast mating assay and *in vitro* by BIAcore and GST-pull down. BIAcore assay allowed us to determine $K_D$ of PTEN/FABP4 complex, which is in the range of 2.78 μM. In addition, we found that PTEN and FABP4 cofractionate in the same high molecular weight complex, when lysates of differentiated NIH3T3L1 cells were analyzed by gel-filtration assay.

***Conclusions.*** It is known that in malignant tumors of prostate and bladder, FABP4 expression is significantly decreased or not detected and it correlates well with the grade of tumor. Moreover, it was also shown that DU145 prostate cell line possesses no FABP4 expression and DU145 could undergo apoptosis when induced to overexpress FABP4 by an ecdysone-controlled expression system. According to this we suggest that there is a functional link between PTEN and FABP4 and this interaction involves PTEN into PPARgamma dependent tumor suppression through, at least in part, terminal differentiation of tumour cells in the adipogenic lineage.