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THE PHENOMENON OF MULTI-DRUG RESISTANCE IN THE TREATMENT OF MALIGNANT TUMORS

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Multi-drug resistance (MDR) is a condition when there is broad cross-resistance of cells to various agents which are different in structure and effect. Modern perceptions on mechanisms of MDR development in malignant tumors have been considered, in particular, in treating breast cancer. Physiological functions and contribution to MDR development of ABC-transporter protein families have been described. The role of activation of glutathione system enzymes and apoptosis-regulating proteins in MDR formation has been shown.

Key Words: **multi-drug resistance, ABC-transporters, glutathione system, apoptosis-controlling genes.**

Modern approaches to treating malignant tumors differ in multimodality and integrity, besides, chemotherapy plays an important role among therapeutic methods.

This treatment option significantly loses its topicality due to the presence of multi-drug resistance (MDR) of tumor cells, as a result of this phenomenon about 90% of treatment cases by cytostatic drugs end up in failures [1]. The MDR phenomenon is a condition, when there is broad cross-resistance of cells to various agents which are different in structure and effect [2, 3]. MDR was first described by the research group of J.L. Biedler in 1970 in experiments with cultivated cells [2]. It was discovered that, when treating cell culture with one drug, a population may be formed which is simultaneously resistant to many other substances, that cells have never contacted (cross-resistance). MDR *in vivo* may both appear during treatment and precede it [4].

Identifying the MDR phenomenon in clinical setting is of special importance, since, to reach the cytostatic effect, one has to either change treatment regimens or carry out aggressive chemotherapy; such treatment doesn't often end up in the necessary outcome and is frequently accompanied by severe complications [5, 6].

MDR is formed by several molecular mechanisms which work both independently and together with one another, however, usually some mechanism is dominant. In forming drug resistance the mechanism providing efflux of chemotherapeutic drugs from the cell comes to the forefront against the concentration gradient with ATP energy consumption, which leads to the intracellular drug level falling below therapeutic concentrations and which is supported by functioning of the ABC-transporter family proteins (ATP-Binding Cassette Transporters) [3, 4, 7–10].

The following mechanisms of MDR development are also topical: firstly, activation of the glutathione system enzymes, which provides tumor cell detoxificating superactivity and promotes fast inactivation of the chemotherapeutic drug or absence of its activation; secondly, mutations of the *p53* gene or activation of *bcl-2* gene, which leads to apoptosis cessation in response to the cytostatic drug action [3, 4, 7].

THE FAMILY OF ABC-TRANSPORTER PROTEINS: STRUCTURE, FUNCTIONS AND THEIR ROLE IN DEVELOPMENT OF THE MDR PHENOMENON

ABC-transporters are a big family of specific transmembrane ATP-binding translocator proteins, carrying various low-molecular weight substrates, including sugars, aminoacids, proteins, metal ions and different hydrophobic compounds [11–14]. In 1993 the research group of M.J. Fath suggested classifying more than 300 proteins of this family into three main groups: eukaryotic transporters, bacterial importers and bacterial exporters [15]. About 50 ABC-proteins have been discovered in a human organism, however, to date it has been proved that genetic evolution of these transporters in vertebrata is still continuing [16]. Studying of the evolution and mutation of ABC-transporter genes is of great importance for modern science, since these proteins influence the functioning of many systems in the organism [11, 12, 16, 17]. Changes in the structure of genes which code transporters are the basis of various pathologies, including disorders in cholesterol and bilirubin metabolism, neurological disturbances, some hereditary diseases etc.; moreover, they lead to a failure in penetration of not only exogenous, but also natural anti-tumor compounds, which are analogues of nucleosides, antimetabolites and tyrosine kinase inhibitors, into the cell [11, 12, 17–19].

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Abbreviations used: BC – breast cancer; BCRP – breast cancer resistance protein; GSH – glutathione; GSTs – glutathione-S-transferases; MDR – multi-drug resistance; MRP – multidrug resistance-associated protein; NBD – nucleotide binding domain; Pgp – P-glycoprotein; TMD – transmembrane binding domain.

ABC-transporters are divided into 7 subfamilies marked as ABCA, ABCB, ABCC, ABCD, ABCE, ABCF and ABCG [13, 20]. The structure of all representatives of the family is quite conservative and is united under domain organization, containing various combinations of two functional subunits: transmembrane binding domain (TMD) or MSD-transmembrane subunit, which consists of six helices, and nucleotid binding domain (NBD) — a subunit including two domains. NBD domains are called Walker A, it is bound to the phosphoric acid residual of the ATP molecule. The second domain — Walker B domain — contains asparagine acid and is bound to magnesium associated with nucleotides [13, 21–23]. All ABC-transporters are divided into full and half transporters according to their structure. Full transporters are characterized by the (TMD-NBD)2 structure and are located in the cell membrane. Transporters with the TMD-NBD structure are called half transporters; they are usually located in the intercellular membranes (lysosomal, mitochondrial membranes, endoplasmic reticulum), the only exception being the ABCG2 half protein (alias BCRP — breast cancer resistance protein), which can be also located in the cell membrane [23, 24].

Prokaryotic and eukaryotic proteins of this family are 30–40% homologous according to the NBD subunit structure, regardless of the transported substrates [22].

In terms of chemical mechanisms, broad substrate affinity of ABC-transporters hasn't been explained for a long time. At present it is supposed that formation of the so-called "inner pocket" underlies the ABC transporter functioning [3, 22, 25]. Such mechanism has been first suggested by Neyfakh, who investigated the functioning of a small bacterium protein, which was named BmrR [26]. The researchers found out that, when binding this protein to the substrate, the alpha-helix of the ligand-binding domain, containing negatively charged gluthamine residue, unwinds (thus, forming "the inner pocket"); after that positively-charged ligands bind to this residue.

In clinical oncology research of expression and activity of ABC-transporters is aimed at eliminating disorder in toxic xenobiotic metabolism in tumor cells, i.e., the MDR phenomenon. From this point of view it is topical to study not all transport proteins, since mutations in genes coding the ABCD subfamily proteins are the basis of solely hereditary peroxisomal diseases [23]. ABCE and ABCF subfamilies include proteins containing one set of TMD-NBD; they are located in intracellular membranes and are not involved in MDR development [17].

Nowadays the ABCA subfamily is the most studied one, however, despite the fact that it includes the biggest amount of human ABC-transporters — 12 representatives — ABCA2 is the only protein of the subfamily that contributes to development of MDR to chemotherapy [23, 27]. Overexpression of ABCA2 in tumor cells gives them resistance to estramustine — an alkylating agent used in chemotherapy of prostate cancer [28].

The ABCB, ABCC and ABCG subfamilies are of the greatest research interest in clinical oncology in terms of MDR development in breast cancer (BC). Contribution of these subfamilies to the development of resistance to chemotherapy has been proved for malignant tumors of various localization: lung cancer, colon cancer, bladder cancer, prostate cancer as well as in leukemia, myeloma and sarcomas [9, 13, 29–39]. Nowadays development of targeted drugs directed to selective inhibition of tumor ABC-transformers is an urgent task [40, 41].

The role of the ABCB subfamily in emergence of resistance to chemotherapy. The ABCB subfamily (another name for the MDR subfamily) includes 11 proteins. Its representatives may be both full transporters (ABCB1 or Pgp, ABCB4, ABCB11), located in the apical part of the cell membrane and half transporters (ABCB2, ABCB3, mitochondrial membrane transporters ABCB6, ABCB7, ABCB8 and ABCB10 as well as lysosomal membrane transporter ABCB9) [23].

The ABCB1 protein (P-glycoprotein, Pgp) is a big transmembrane protein with the molecular weight of 170 kDa; it is the largest studied representative of the ABC-transporter family which is involved in developing resistance of tumor cells to chemotherapy. Since the interrelation between membrane transporter proteins and MDR was first demonstrated when studying P-glycoprotein, MDR, determined by overexpression of the stated protein, is also called typical or classic [3]. The protein was discovered in the membrane of resistant cells in the research laboratory by the research group of Juliano and Ling in 1976 [42].

Human Pgp is coded by the *MDR1* gene, located on the chromosome 7 (7q21.1); besides, resistance development may follow both a change in *MDR1* gene expression and amplification of the genome part containing the *MDR1* gene and five-six other genes coupled with it [43–45]. Mechanisms of resistance development were studied in some research on investigating specially selected resistant Cerb-1 and Cerb-2 sublines as well as the CHLV-79RJK line of Chinese hamster cells [43, 44].

The *MDR1* gene has two promoter regions — lower and upper promoters; gene activity is mostly regulated by the lower promoter [46]. Transcription activity of *MDR1* rises in response to different impacts, including the effect of chemotherapy drugs (also the ones that are not Pgp substrates) [47]. Moreover, *MDR1* activity may be influenced by p*53, ras, raf, RAR-alpha* and *RAR-beta* genes (genes of retinoid acid receptors) [3, 48] as well as genes of c-*fos* and *c-jun* transfactors [3, 49]. Supposedly, protein kinase C, protein kinase A and other protein kinases also take part in regulation of Pgp activity [50]. Taking into account multi-activity of MDR1/Pgp, it may be concluded that this transport system is extremely necessary for normal cell functioning; that is why its regulation is more than once repeated [3].

Pgp is a polypeptide which consists of two similar parts. Each part includes six hydrophobic transmem-

brane regions which cross the cell membrane 12 times, thus, forming pores and canals, by means of which Pgp releases substances, using ATP hydrolysis energy from two binding sites in NBD domains [3, 25]. When dyed by monoclonal antibodies, predominant ABCB1 localization is found on the surface of the cell membrane; small amount of ABCB1 are located in the area of Golgi complex [3, 51].

Prominence of Pgp expression in different tissues is not even; high values are found in tissues which actively contact xenobiotics: liver, kidneys, mucous membranes of the large and small intestine, adrenal tissue, brain capillary endothelium, testicles, placenta trophoblast [32]. Extremely low level of protein expression is also found in the lungs and epidermis cells. In the bone marrow CD34-positive cells express functionally active Pgp [50], then, as cells of the myeloid type become mature, the expression and functional activity of this protein gradually decrease [35]. Tumors originating from Pgp-expressing cells will have pre-existing MDR.

The physiological role of ABCB1 is to protect from xenobiotics and export of endogenous metabolites [52, 53]: presence of P-gp in the intestinal epithelium prevents toxic substances entering the organism; its expression in renal tubules and the canalicular membrane of hepatocytes ensures their elimination from the organism. Pgp is overexpressed in the epithelium of the brain choroid plexus, which indicates its role in forming the blood-brain barrier [54]. P-glycoprotein located in the placenta trophoblast not only plays a significant role in limiting the effect of drugs and toxins on the fetus, but it is also necessary for normal development of the placenta and for maintaining its physiological functions [52].

Pgp is to a different extent expressed in all types of tumors. Tumors that are characterized by overexpression of the *MDR1* gene are carcinomas of the large intestine and kidneys, hepatomas, renal tubule cancer, pheochromocytomas. In adenocarcinomas of the lungs and testicles, BC and sarcomas emergence of acquired MDR has been proved, which is determined by a rise in Pgp expression during chemotherapy or which is associated with genomic instability of malignant cells [55–58].

Pgp enhances tumor resistance to taxanes, podophyllotoxins, alkaloids of plant origin, including vinca alkaloids, antracyclic antibiotics and other chemotherapy drugs. Besides, this protein ensures fast elimination of many other substances: ethydium bromide, fluorescent dyes, gramicidin D, puromycin etc. [3, 44].

Pgp-mediated MDR is characterized by a decrease in cell resistance to chemotherapy drugs under the effect of MDR modulators or inhibitors [59]. Inhibitors are membrane-active low molecular weight lipophilic substances similar in their chemical compositions: calcium channel blockers (CCB) (nifedipine, verapamil and its analogues), trifluoroperasine, quinine, representatives of the acridine group, indole alkaloids (including reserpine and yohimbine), some antibiotics (cephalosporin, gramicidin, puromycin) and other [1, 50, 60]. Highly-efficient inhibitors discovered in the last years are A PSC833 (the analogue of cyclosporine) and the carboxamide derivative GG918 [60].

One of the mechanisms of inhibitor action is competition with cytotoxic agents for binding to Pgp (for example, in case of verapamil). The problem lies in the fact, that all the above-mentioned MDR modulators have been discovered in experiments *in vitro* in cell cultures, their use in clinical practice is impossible at this stage. The research has shown that for the majority of inhibitors it failed to obtain the concentrations in blood of patients that are necessary to overcome drug resistance, since a rise in dose gives severe complications [60].

In recent years new therapeutic approaches have been actively searched, such as the use of monoclonal antibodies to Pgp as MDR modulators and regulation of ABCB1 expression with help of epigenetic modulators (for instance, inhibitors of histone deacetylases — HDACi); nowadays clinical trials are being carried out [56, 61].

The role of the ABCC family in emergence of resistance to chemotherapy. This group includes 13 representatives which are full transporters; the majority of them (ABCC1–6, 10, 11) are active transmitters of various organic anions in hydrophobic drugs, therefore, they are involved in MDR development [11, 12].

The ABCC7, ABCC8 and ABCC9 proteins don't participate in MDR development, however, they perform important functions of sustaining normal cell metabolism. The ABCC7 protein (CFTR) functions as an anion channel; it regulates their flow in epithelium cells. In case of mutations in the gene coding this transporter, cystic fibrosis develops [11, 12]. The ABCC8 (SUR1) and ABCC9 (SUR2) proteins, which are receptors of sulfonylurea [62], regulate permeability of ATP-specific potassium channels (Kir 6.2 and Kir 6.1, respectively). Mutations in genes that code these proteins may serve a reason for decreased tolerance to glucose [63].

The thirteenth family representative — ABCC13 — was discovered most recently; it was cultivated from cells of the liver, fetus and placenta, and its structure and functions are yet to be studied [11, 12].

A group of the ABCC family proteins, which determine MDR development, is united under a common name — multidrug resistance-associated protein (MRP) and includes nine members. MRP are transporters of organic anions (except for MRP9, which function has not been identified yet); consequently, they transport anionic drugs, such as methotrexate, and neutral drugs conjugated to acid residues, such as glutathione, glucuronate and sulfate. However, MRP1, MRP2, MRP3 may also cause resistance to neutral organic drugs, which don't form conjugates to acid residues [11, 12, 33, 64].

Six main representatives of MRP proteins (MRP1–6) are divided into two groups. MRP1, MRP2, MRP3 and MRP6 have the external N-terminal domain (marked as TMD_0), which Pgp lack. MRP4, MRP5 don't have the TMD_0 domain, the degree of similarity of these

proteins to MRP1 is less than 40% [33]. Nevertheless, they are more homologous than P-gp or other classes of ABC-transporters. Besides, research has shown that TMD_0 is not necessary for transport activity [65].

The most well-studied representatives of MRP are the ABCC1 and ABCC2 proteins. The ABCC1 protein or MRP1 was discovered in 1992 by Cole et al. in lung cancer cell lines resistant to doxorubicin [72]. It is a transmembrane protein located on the basolateral cell surface. Preferable substrates for MRP1 are organic anions, i.e., drugs conjugated to glutathione, glucuronate or sulfate [31, 34]; it is one of the main transporters of glutathione conjugates [66]. It is a protein with the molecular weight of 190 kDa, which gene is located on the chromosome 16 (16p13.1). It has five transmembrane segments in MSD2 and four-six transmembrane segments in MSD3 [67, 68].

MRP1 is widely expressed in epithelial cells of the organism, including digestive, urogenital and respiratory systems, in endocrine glands and hematopoietic organs, including all peripheral blood cells, regardless of their differentiation [30, 33, 36, 39]. Some researchers consider overexpression of the protein in bronchial epithelium as one of the reasons for bronchial asthma development [36].

High expression level of this protein has been detected in many tumor cells in lung cancer, colon cancer, BC, bladder cancer, prostate cancer as well as in leukemia [30, 69–71]. MRP1 plays an important role in cell MDR to etoposide, antracyclic antibiotics, vinca-alkaloids, stibium and arsenium drugs [72] as well methotrexate [33, 73].

Nowadays effective and safe MRP1 modulators are being searched. As potential MRP inhibitors LTC4, LTD4 and S-decylglutathione [74] are considered along with organic acids, originally developed for suppression of uric acid transport: sulfinpyrazone, benxbromarone and probenecid [75].

The ABCC2 (MRP2, cMOAT, cMRP) protein was first discovered in the hepatocyte cell membrane; due to this fact it was named canalicular multispecific organic aniontransporter (cMOAT) [76]. MRP2 is a transporter of bilirubin glucuronides in bile; gene mutations or a decrease in expression of this protein are known as the Dubin — Johnson syndrome [77].

ABCC2 expression is also identified in cells of proximal kidney tubules and intestinal epithelium. The *MRP2* gene is located on the chromosome 10 (10q24). According to its domain organization it resembles *MRP1*; it is also a transporter of substances conjugated to glutathione, glucuronic and sulfuric acids [13].

At present active research is being carried out on studying the link between MRP2 expression and the course of BC. By the immunohistochemical analysis it has been shown that two types of ABCC2 proteins are identified: the one located in the cell membrane and tumor cell cytoplasm (ABCC2c) and the one located in nuclear envelope of atypical cells (ABCC2n). According to the research results it may be concluded that tumors with overexpression of the protein in both locations are characterized by a worse forecast. If the protein is located in the nuclear envelope, tumors have more aggressive clinical presentation of the disease compared to cases when ABCC2 are located on the cell membrane [29].

MRP2 may be the cause of tumor cell resistance to methotrexate, cisplatin, etoposide, doxorubicin, epirubicin, vincristine and mitoxantrone [29, 72, 78]. On the whole the range of resistance coincides with the one in MRP1 with one exception: MRP2 induces resistance to cisplatin, which is not observed in MRP1 [78, 79].

Now effective inhibitors of ABCC2 are being searched; though MRP1 and MRP2 have similar substrate specificity, MRP1 inhibitors are not necessarily effective with respect to MRP2; for example, sulfinpyrazone is applicable only for ABCC1 therapy [33].

ABCC3 (MRP3, MOAT-D, cMOAT-2, MLP-2) is also a transporter of organic anions; however, as opposed to MRP1 and MRP2, it prefers glucuronates to glutathione conjugates [45, 80]. The *MRP3* gene is located on the chromosome 17 (17q21.3), it codes protein expressed in norm in the epithelium of the large and small intestine, hepatocytes, pancreas, adrenals and lungs [11, 12, 64].

When studying the cell culture and the histological material from patients with lung cancer, MRP3 expression determines a decrease in sensitivity to etoposide, doxorubicin, vincristine and cisplatin. In the meantime, no direct link between MRP3 overexpression and tumor prognosis has been observed [81]. These cells are also resistant to short-term effect of methotrexate [82].

ABCC4 (MRP4, MOAT-B) is a specific transporter of conjugates to phosphorous acid residues (analogues of nucleosides); but it can also transport glutathione, glucuronides and sulfates. The gene coding MRP4 is located on the chromosome 13 (13q32). The MRP4 structure differs from that of MRP1, MRP2 and MRP3 in the absence of the NH_2-terminal domain MSD (TMD_0) [79]. Schuetz et al. [83] have found out that MRP4 eliminates drugs for HIV treatment from the cell: 9-[2-(phosphono metoxi)ethyl] adenine (PMEA) and azidothymidine monophosphate (AZTMP) in cells that are resistant to PMEA. High level of MRP4 is also a reason for severe drug resistance of cells to some nucleoside analogues, including 9-[2-(phosphono metoxi)ethyl] guanine, which has antitumor activity. Moreover, tissues with MRP4 overexpression have short-term resistance to methotrexate, as MRP3 [84]. Lai et al. have established that glutathione plays an important role in MRP4 functioning; depletion of its intracellular reserves decreases cell resistance to nucleoside analogues [85].

The gene coding ABCC5 (MRP5, MOAT-C, pABC11, sMRP) is located on the chromosome 3 (3q27), high expression of this transporter has been detected in many tissues of the organism, especially in the musculoskeletal system and in the brain [79]. Overexpression of MRP5 leads to resistance to thiopurines, such

as 6-mercaptopurine and tioguanine, which are used to treat acute lymphoblastic leukemia and acute myeloid leucosis, and to PMEA [33]. McAleer et al. [86] also found that accumulation of anionic fluorochromes decreases in MRP5-transfected cells; they acquire resistance to heavy metals. Research has demonstrated that MRP5 may be inhibited by sulfopyrasone and benzbromarone [87].

The *ABCC6* gene (MRP6, MOAT-E, MLP-1, ARA) is located on the chromosome 16 (16p13.1); high level of MRP6 expression has been detected in the liver on the lateral hepatocyte membrane and in the kidneys [82]. The physiological role of this protein is unclear. To date it has been known that deletions and other mutations in the gene, coding this protein, lead to development of pseudoxanthoma elasticum, a disease that is characterized by disorder in formation of yellow fibers accompanied by skin, eye and cardiovascular lesions [88].

Belinsky et al. [89] have analyzed the contribution of MRP6 to MDS: in MRP6-transfected cells low level of resistance to anthracyclic antibiotics and podophyllotoxins (etoposide, tenyposide) is observed.

Interestingly, overexpression or full/partial amplification of the *MRP6* gene was found only in cell lines with MRP1 overexpression/amplification. It is likely that MRP6 doesn't play a significant role in tumor cell resistance, and its expression together with MRP1 is explained by close proximity of their genes on the chromosome 16 [82, 90]. Amplification of 3-end of the MRP6 protein is of great importance in cell MDR of patients with acute myeloid leukemia; earlier this MRP6 region was called ARA (anthracycline resistance-associated protein) [91].

ABCC10 (MRP7) is a protein with the molecular weight of 158 kDa, coded by the gene located on the chromosome 6 (6p12–21). It has been discovered relatively recently in reticulocyte lisates; its extremely low expression was detected in other tissues as well; its physiological role is currently unknown [92].

ABCC11 or MRP 8 is a protein with the molecular weight of 150 kDa, which gene is located on the chromosome 16 (16q12.1). In normal tissues moderate protein expression is observed in the testicles and the liver. The MRP8 structure is close to that of MRP5: homology is 42% [93].

The research by Bera et al. [94] determined high level of MRP8 expression in tumor tissues of patients with BC. The MRP8 gene codes two different RNA transcripts: 4.5-kb and 4.1-kb. In the meantime, 4.5-kb transcript was detected in breast tumors, whereas 4.1-kb — in testicle cells. The physiological role and the contribution of this transporter to development of BC as well as resistance to pharmacotherapy is currently unknown [93, 94].

The ABCC12 (MRP9) protein was identified by two research groups — Bera et al. [94], Tammur et al. [93] — in tumor cells of patients with BC. The *MRP9* gene, located on the chromosome 16 (16q11), also codes two different mRNA transcripts (4.5-kb and 1.3-kb transcripts), which are expressed in various tissues in a different way: 4.5-kb transcript was detected in tumor tissue in BC and in normal tissues of the breast and the testicles, whereas the 1.3-kb transcript was found in brain tissues, skeletal muscles and testicles [95]. The physiological role of MRP9 and its contribution to MDR emergence are currently not determined.

The role of the ABCG family in emergence of resistance to chemotherapy. Five human proteins of this group are described; they are represented by half membrane transporters located in the cell membrane. This differentiates them from other half transporters situated in intracellular membranes [96].

It is proved that mutation of genes that code ABCG5 and ABCG8 leads to development of rare autosome-recessive pathology — p-sitosterolemia, which is characterized by accumulation of plant sterol in blood and tissues [97].

The main physiological role of the ABCG1 protein is support of lipidic homeostasis in organism tissues, especially in the lungs, central nervous system and vessels [96, 98–100]. Besides, this protein participates in secretion of insulin by pancreas β-cells [98, 100–102], controls proliferation of hematopoietic stem and multipotent precursor cells both in the bone marrow and spleen in case of extramedullary hematopoiesis [100, 103]. ABCG1 malfunction leads to development of atherosclerosis, excess accumulation of lipids in macrophages and formation of foam cells [98, 99, 104]. Mutations of the gene coding the ABCG1 protein are accompanied by risk of type II diabetes development [101, 102]. The role of this protein in MDR development is unclear, however, in 2012 V. Hlaváč et al. compared breast tissue samples before and after neoadjuvant therapy and found out that after therapy expression of ABCG1 rises [105].

The ABCG2 protein has been described to the fullest extent, it is the only representative of the family, which overexpression is proved to cause MDR development [96]. This protein was first discovered by the research group of L.A. Doyle in 1998, when studying the MCF-7 cell line of BC; it was named BCRP. The stated protein was also identified in other research, it has interchangeable names MXR (mitoxantrone resistance protein) and ABCP (placental ABC transporter) [106–110].

BCRP is a protein with the molecular weight of 72.6 kDa, its coding gene is located on the chromosome 4 (4q22) [111]. High expression of this protein is marked in the placenta, myocardium, testicles and endothelium of vessels [111, 112]. Medium level of expression is found in the liver, large and small intestine, brain and prostate; low expression during histochemical studies has been discovered in the lungs, skeletal muscles, pancreas, kidneys, spleen, thymus, multilayered skin and esophagus epithelium and peripheral blood leukocytes [106, 112, 113].

Physiological functions of BCRP lie in providing tissue protection from endogenic toxins or xenobiotics and

regulating cell homeostasis of physiologically important compounds such as heme, porphyrin, riboflavin and estrogens [114–116]. ABCG2 plays the key role in folic acid homeostasis, which may promote cell survival in conditions of hypoxia [108]. Besides, much research has shown that high expression of this protein is marked in primitive stem cells of human bone marrow, which ensures protection of these cells from xenobiotics [109].

Research of BCRP expression in normal tissues that was carried out by Doyle and Ross [113] proved an extremely high level of ABCG2 in the placenta (in particular, in syncytiotrophoblast and on the apical surface of chorionic villi) — about 100 times higher than in other tissues with high expression of this protein. This fact suggests that BCRP plays a particular role in protecting the fetus from toxic substances [52].

Many researchers believe that this transporter performs a protective function as part of the blood-brain barrier due to high expression in the endothelium of brain vessels [106–108, 117].

In tumor cell lines a high level of ABCG2 has been detected in BC, colon cancer, stomach and lung cancer, myeloma and sarcomas [13, 118–120]. ABCG2 of mRNA has also been identified in blast cells of patients with acute myeloid leukemia resistant to chemotherapy [121, 122].

It has been proved that overexpression of BCRP promotes resistance of tumor cells to mitoxantrone, antracyclic antibiotics (in particular, doxorubicin), methotrexate, camptothecin derivatives (topotecan, SN-38) and indolocarbazole derivatives. In the meantime, resistance to antracyclic antibiotics is unstable and is probably connected with the presence of mutation in the 482 codon of the gene coding BCRP [109, 113, 123–126].

At present effective specific inhibitors of BCRP are being searched, the influence of tyrosine kinase inhibitors as well as a number of antiviral drugs, used for HIV treatment, statins and imatinib on the transporter function is studied [106, 109, 124]. At the moment a whole class of new compounds synthesized from XR9576 (tariquidar) has been received, which selectively inhibit BCRP [110].

THE ROLE OF ACTIVATION OF GLUTATHIONE SYSTEM ENZYMES IN EMERGENCE OF MDR

Glutathione (GSH) is a tripeptide which is synthesized in the organism from glutamic acid, cycteine and glycin; it performs detoxificating, antioxidant and immunoprotective functions. The action of glutathione is ensured (carried out) by its conjugation in various electrophilic substances; this process is catalyzed by glutathione-S-transferases (GSTs) [127, 128].

GSTs are found in all mammals and plants. They may exist as homo- and heterodimers with the molecular weight of 43–57 kDa, each subunit has its own independent binding site, which, in turn, contains two subsites: G and H. The former interacts with glutathione (contains protein –SH group, histidine and arginine); the latter binds the hydrophobic substrate, it contains glycine. According to identity of the aminoacid composition, GSTs in mammals are grouped in six classes: α- (alpha-), μ- (mu-), κ- (kappa-), θ- (theta-), π- (pi-) and σ- (sigma-) GST. In human organism mostly four main GST classes are expressed: GSTA (α-class), GSTM (μ-class), GSTT(θ-class) and GSTP(π-class) [127, 129]. Glutathione transferases are mainly located in the cytosol and endoplasmic reticulum, however, they may be found in nuclei and mitochondria as well.

Representatives of the GSTA class are widely expressed almost in all tissues of the organism; they are the main representatives of glutathione transferases in the liver. The GSTM class includes five genes with different expression localization: GSTM1 is expressed in blood and liver cells, GSTM2 — solely in muscles, GSTM3 and GSTM4 — in cells of the central nervous system, GSTM5 — in cells of the central nervous system, liver, testicles and to a smaller extent — in transverse cardiac muscles. Glutathione transferases of the theta-class are located in erythrocytes and cells of the liver. The GSTP class, as the only representative of GSTP1, is also widely spread in cells of the organism as well as alpha-class representatives, but it lacks in erythrocytes; its highest expression is marked in cells of the placenta and skin [127, 129, 130].

Recently it has been discovered that the GSTs system not only promotes detoxification of xenobiotics and cancer-inducing substances, but also acts as a modulator of the pathway which transmits signals that control cell proliferation and apoptosis [127, 130].

Multifunctional family of glutathione-S-transferase also plays an important role in catechol estrogen metabolism. In case of catechol estrogens GSTs act as a detoxificating agent, promoting conjugation of genotoxic estrogen metabolites to glutathione, which, as well as conjugation to N-acetylcycteine and cycteine, causes their inactivation [131–134]. In some research it has been shown that there is a link between GSTs gene polymorphism and risk of BC development. In the so-called zero genotype of GSTs, which is characterized by low enzyme activity, risk of BC development was 2.1 times higher in premenopausy and 2.5 times higher in post-menopause, and in case of zero genotype combined with low activity of catechol-O-methyl transferase it was 3.5 times higher [132, 135].

Disorders in glutathione system metabolism in the human organism may be the cause of malignant tumor development. A rise in GSTs activity in tumor cells results in development of resistance to a number of chemotherapy drugs [136]. Thus, according to the carried out research, high concentration of glutathione has been detected in cell lines resistant to alkylating agents (embichine, chlorbutin, melphalan, cyclophosphamide etc.) and other drugs [3, 132, 137, 138].

The role of glutathione system activation in development of drug-resistance was demonstrated when studying the level of GSH and GSTs in P 388 leukemia cells resistant to cycloplatam. The level of glutathi-

one was almost 10 times higher compared to parent cells of this line, whereas glutathione transferase activity was 1.5 times higher in cells of the resistant strain as opposed to the sensitive one. Cycloplatam administered to tumor carrying mice has caused a significant increase in the level of GSH in tumor cells of both strains. The obtained results indicate that GSH-dependent enzymes may significantly contribute to drug resistance of cells [139]. Thus, the role of MDR development associated with glutathione transferase activation in brain tumors has been proved [140].

When studying changes in the glutathione system, emergence of resistance has been proved not only to alkylating agents, but also to antracyclic antibiotics and vincristine (drugs of Pgp-MDR), which suggests existence of general mechanisms of regulation of ABC-transporter genes and genes involved in glutathione metabolism [139].

The research group of C.S. Morrow [141] has shown that overexpression of PGP and a rise in GST activity are identified in the MCF7 cell line of BC resistant to 4-nitroquinoline-1-oxide (4-NQO). In the meantime, such combination is associated with GST-dependent increase in formation of 4-NQO-glutathione-conjugates, which are eliminated from cells by Pgp [141, 142].

Many types of malignant tumors contain the increased amount of glutathione in tissues and have high activity of glutathione transferases, which allows their cells to increase resistance to chemotherapy right up to MDR formation. At present a number of drugs inhibiting GSTs selectively in tumor cells are being developed; their use in combination with cytostatic drugs should increase the efficiency of anti-tumor therapy. Inhibitors are also considered as one of the potential therapeutic approaches to treating other diseases related to aberrant cell proliferation [143, 144].

THE LINK BETWEEN CHANGES IN APOPTOSIS-CONTROLLING GENES AND EMERGENCE OF MDR

Nowadays the mechanism of MDR development related to apoptosis suppression is paid more and more attention to, since the cytostatic effect of the majority of drugs is realized by induction of this very pathway, regardless of a certain mechanism of action of every drug. The key role in apoptosis is played by functional activity of *TP53* and *bcl-2* genes as well as their protein products [7, 145–149].

Mutation of the TP53 gene and development of the MDR phenomenon. During cell tumor transformation and tumor progression one of the most common changes is mutation of the TP53 tumor in the suppressor gene [150–152]. In norm the *TP53* gene is activated in response to various cell damaging factors; in the meantime, the wild type of the p53 protein is synthesized, which leads to a stop of the cell cycle and (or) facilitates cells entering apoptosis [153, 154]. In defective cells conditions for damaged DNA reparation emerge, otherwise cells are removed from the population. Cells where normal p53 lacks are resistant to apoptosis induction [155]. The p53 mutagenic protein demonstrates the properties of an oncogene, since it doesn't have the ability to stop cell division at the key points of the cell cycle, which results in the beginning of DNA replication in cells on the damaged matrix. It promotes instability of the genome and development of malignant transformation [156, 157]. Therefore, tumor cells with mutant TP53 are resistant to the action of alteration factors, which include cytostatic drugs [3, 158].

It has been proved that stromal cells of malignant epithelial tumors, in particular, fibroblasts with unchanged suppressor gene *p53*, are able to produce and secrete tumor inhibiting factors [159, 160].

Inductors causing the wild type of p53 are a combination of agents that cause DNA damage in different ways, for example, by changing the redox-potential (in particular, during accumulation of reactive oxygen species) or the nucleotide pool in the cell, which leads to destruction of the mitotic spindle of cell division etc. [153, 161]. Due to this fact malignant tumors with the mutant p53 are typically resistant to drugs with different mechanism of action (for instance, to cisplatin and 5-fluorouracil) [3].

There are also many facts proving that the p53 protein participates in regulation of ABCB1 action. It has been shown that the wild type of p53 suppresses and the mutant p53s protein increases ABCB1 activity [48, 56, 158]. Besides, when studying MDR of rhabdomyosarcoma and neuroblastoma cell lines, it has been found out that p53 is a strong inhibitor of P-glycoprotein and ABCC1 (MRP1) functions [56, 57]. When studying MDR of prostate cancer, the correlation between a decrease in the degree of tumor differentiation and a rise in MRP1 expression has been identified. A combination of these two mechanisms resulted in development of tumor resistance to doxorubicin and vinblastine [57, 162, 163].

Therefore, functional recovery of the wild type of p53 may be the strategy for reducing or preventing MDR; it may also help increase sensitization of cancer cells to anti-tumor drugs [56, 153, 154, 164, 165].

The Bcl family of proteins and its influence on emergence of resistance to chemotherapy. Besides tumor suppressors, malignant tumor sensitivity to various xenobiotics is influenced by antiapoptotic proteins. The leading role in this process is given to the Bcl family proteins [166]. Functions of proteins of this family are directly opposite to one another; the *bcl-2* gene codes proteins having both antiapoptotic (BCD-XL, Bag, Bcl-2) and proapoptotic (Bcl-xS, Bax, Bad, Bak, Bid) effects on cells [133, 167, 168].

It was proved that during Bcl-2 overexpression tumor cells have drug resistance to various cytostatic drugs [169]. There are data indicating that expression of the bcl-2 gene may be referred to markers of drug resistance to chemotherapy in a number of malignant tumors: bladder cancer, prostate cancer, testicular cancer, myeloid and lymphoid tumors of the hematopoietic system, brain tumors [130, 170, 171].

Clinical studies have detected the interrelation between the expression level of antiapoptotic genes bcl-2 and bcl-xL in various tumors and their high resistance to chemotherapy drugs [172, 173]. However, in lung and BC one can't say for sure about the correlation between bcl-2 overexpression and resistance to chemotherapy, since expression of antiapoptotic proteins Bc1–2 and Bcl-xL occurs in cells of these tumors alongside with expression of the Bax proapoptotic protein [145, 173–177]. The Bc1–2 and Bax proteins form heterodimers inactivating the action of the antioncogene; consequently, the proportion of these proteins in the cell determines its predisposition to apoptosis and chemotherapy drug action [145, 176]. In the work by Sumantran et al. intensification of induced apoptosis has been detected in the transfected cell line of BC MCF-7 in response to etoposide and taxol [178]. According to the latest research, overexpression of Bcl-2 that occurred after first-line chemotherapy of BC suggests MDR development in response to therapy [55, 173, 179, 180].

Proapoptotic proteins bcl-2 have a domain in their structure called BH3, its binding by antiapoptotic proteins bcl-2 leads to apoptosis suppression [181]. Compounds that inhibit this bound induce apoptosis on their own or in synergism with other cytostatic chemotherapy drugs. Recently a whole range of such drugs has been developed, however, further research has shown that many assumed inhibitors are not specific and have other cell targets [181–184]. There are two exceptions to this: for instance, ABT-737, its successful application as the only drug agent for treatment of leukemia and lung cancer has been proved. The second drug is ABT-263; at present it is at the stage of clinical trials for treating small-cell lung cancer, lymphatic leukemia and lymphoma [185, 186].

In conclusion it should be said that disorder in regulation of genes that are involved in apoptosis control (oncosuppressor p53 and genes of the bcl-2 family) may lead to cells developing resistance to a wide range of anti-tumor drugs — DNA-tropic agents (adriamycin, actinomycin D), antimetabolites (5-fluorouracil), but not to the compounds interacting with mitotic spindle of cell division (colchicine, taxole) [187–191].

CONCLUSION

Nowadays one of the leading methods of treating malignant tumors is chemotherapy, both non-adjuvant and adjuvant. This therapy is significantly losing its topicality due to the presence and (or) development of MDR. In the mechanism of MDR development activation of ABC-transporter proteins and glutathione system enzymes as well as changes in apoptosis-controlling genes plays the key role. Therefore, determination of MDR marker expression in malignant tumors will allow to assess the rationality of chemotherapy prescription and correct the treatment strategy. Due to this fact it is topical to study markers of MDR depending on the morphological structure and molecular-genetic characteristics of the primary tumor.

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