MDR1 gene polymorphisms and P-glycoprotein expression in respiratory diseases

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Background. P-glycoprotein (P-gp/*MDR1*), a member of the ATP-binding cassette (ABC) transporters super family, encoded by the *ABCB1/MDR1* gene, is one of suggested respiratory tract protection components, found in various tissues with a barrier function, such as tracheobronchial epithelium and lung parenchyma. As an ATP-dependent pump, P-gp extrudes lipophilic particles out of cells and acts as a gatekeeper against numerous xenobiotics, with a protective role in mediating DNA damage, secretion of toxic compounds, apoptosis and the immune response. Therefore, a presence of *MDR1* polymorphisms and altered P-gp expression may be important for pathogenesis of reduced lung inflammatory response on cigarette smoke exposure, as well as for the severity of chronic obstructive pulmonary disease and lung cancer pathogenesis and treatment efficacy.

Methods and Results. We have analyzed data available from experimental and clinical studies performed to establish the role of *MDR1* polymorphisms, especially the 3435C>T variation, and P-gp expression in pathogenesis and clinical outcome of human respiratory diseases.

Conclusions. Although there are indications that altered expression of P-gp and/or polymorphisms of *MDR1* gene play an important role in respiratory diseases pathogenesis and treatment, their exact role and relevance are insufficiently investigated, with exception of certain chemotherapeutic agents' efficacy in lung cancer treatment. Further research in this field, including bigger series of patients, is necessary for better understanding of respiratory diseases' pathogenesis and treatment.

Key words: P-gp expression, *MDR1* polymorphisms, respiratory diseases, COPD, lung cancer

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INTRODUCTION

Human respiratory system is continuously exposed to environmental pathogens, irritants, pollutants and agents which produce oxidative stress. Therefore, the existence of various defense mechanisms throughout respiratory system is essential. At the cellular level, one of the most important protective mechanisms is to pump toxins out of cells, with help of transporting protein systems commonly found in the cell membranes of many organisms, from bacteria to mammals, responsible for cell protection. Many multidrug resistance protein (MDR) group members, present in virtually every cell of all species, can act as drug efflux pumps, resulting in decreased intracellular concentrations of toxic compounds at the site of action¹. The prominent expression of P-glycoprotein (P-gp, product of *MDR1* gene) and other MDR proteins throughout respiratory tract indicate that these transporters may be important for protection against endogenous or exogenous toxic compounds entering the lungs². However, this protection seems to reverse if some change in genetic structure of *MDR1* gene is present, or when P-gp expression is altered. These changes affect the natural protection of respiratory tract by accumulation of toxic compounds (i.e. tobacco smoke constituents) when P-gp activity is reduced. Furthermore, if P-gp is overexpressed, xenobiotics

are metabolized rapidly and expelled out of cells, which is especially important for lung cancer treatment, leading to the reduced efficacy of chemotherapeutic drugs used in lung cancer patients.

Human *MDR1* gene structure and variations

The multi-drug resistance-1 (*MDR1*) gene is located on human chromosome 7 and encodes a 170-kDa plasma membrane glycoprotein (P-gp), a member of the ATP-binding cassette (ABC) transporters super family, also known as *ABCB1*. The *MDR1* gene is composed of 28 exons ranging in size from 49 to 209 base pairs (bps) encoding an mRNA of 4.5 kb. Polymorphisms of *MDR1* gene were first described by Hoffmeyer et al. who found a correlation between lower intestinal expression of P-gp and a polymorphism in exon 26 (ref.³). Initially, only 15 different SNPs were found in the exons of *ABCB1*, later increasing to total of at least 50 SNPs and insertion/deletion polymorphisms identified^{4,5}. Many of these SNPs are silent (synonymous), and do not produce a change in amino acid sequence. Studies of different ethnic groups suggested that polymorphisms found in *ABCB1* denoted as 1236C>T, 2677G>T/A/C, and 3435C>T, are the most common. The C3435T single nucleotide polymorphism located in exon 26 of the *MDR1* gene was shown to be associated with P-gp levels and substrate uptake³. Individuals

who were homozygous for the T-allele had a significantly decreased P-gp expression level compared to those homozygous for the C-allele. ABCB1 synonymous SNPs 1236C>T and 3435C>T are linked to a non-synonymous SNP (2677G>T, Ala893Ser) (ref.⁶) and occur with slightly different, but similar frequency in European populations, while their presence is statistically significantly different in ethnic groups of African and Asian origin⁷.

Structure and expression sites of P-gp

The human *MDR1* mRNA encodes P-gp, a polypeptide with 1280 amino acids, also known as ABCB1. The protein is defined as having two halves, each containing six hydrophobic trans-membrane domains, and an ATP binding domain. The two halves are separated by a flexible linker region, and the two ATP-binding domains are structurally similar. All 12 trans-membrane domains are found in the plasma membrane⁸. P-gp is expressed in the apical membrane of cells with excretory functions, such as those in the liver, kidney, small intestine, stomach, and the blood-brain barrier^{9,10}. The physiological expression of P-gp in tissues is an important determinant for drug detoxification in various cells and tissues. This protein also mediates the transfer blocking of hydrophobic xenobiotics across the placenta and prevention of the entry of substrates into the central nervous system as a part of the blood-brain barrier¹⁰. A novel investigation has recently shown that P-gp is also expressed within cells and localized to lysosomes, allowing P-gp mediated lysosomal sequestration of xenobiotics¹¹.

Genetic variants of *MDR1* can influence interindividual variability in the bioavailability and pharmacokinetics of various drugs^{12,13}. Some findings suggested that 3435C>T was associated with significantly reduced intestinal P-gp expression in T/T homozygotes in comparison with subjects homozygous for C allele (C/C), leading to higher steady state plasma concentrations after the oral administration of digoxin^{13,14}. However, a large number of the subsequent human studies shown the inconsistent observations even when the same drugs were tested, in the same disease and ethnic group^{14,15}.

MDR1 gene, P-gp and lungs

Tissues with a barrier function, such as lung parenchyma and tracheobronchial epithelium, have high transcriptional activity for many ABC transporters^{16,17}. In human lung, P-gp is expressed at the apical side of ciliated epithelial cells or ciliated collecting ducts, and on apical and lateral surfaces of serous cells of bronchial glands but not in mucus-secreting goblet cells¹⁸. Epithelial cells of the trachea and major bronchi stained strongly for P-gp while staining of the smaller bronchi is patchy or absent. P-gp was also found in the lateral membranes of normal nasal respiratory mucosa¹⁹. In human and rat type I epithelium, P-gp is located at the luminal side whereas freshly isolated type II cells lack P-gp²⁰. In study performed by Cordon-Cardo et al., pneumocytes did not stain for P-gp²¹. Some antibodies visualized P-gp in endothelial cells of blood vessels^{18,21}. Alveolar and blood monocyte-derived macrophages stained positive but variably for P-gp². The

observed apical epithelium expression may signify that P-gp is important for transport of compounds from the interstitium into the lumen, although the precise function of P-gp in the lungs remains unknown.

Animal models

Rodents contain two *Mdr1* genes, denoted as *Mdr1a* and *Mdr1b*. Scheffer et al. reported high P-gp levels in lungs of mice². In mice orally treated with dexamethasone for 24 h, *Mdr1b* mRNA expression in lungs was decreased, and that finding was a base for speculation that dexamethasone treatment of lung tumors may reverse MDR (ref.²²). In the lung tissue of *Mdr1a/1b* (-/-) mice²³, the level of [3H]digoxin was rather low compared to brain, ovary and adrenal glands, and it was measured 2.6 times higher in (-/-) mice than in (+/+) mice, but this was not significant.

In research by van der Deen et al., triple knock-out (TKO) mice lacking the genes for *Mrp1* and *Mdr1a/1b* were more susceptible to develop COPD features than the wild-type (WT) experimental animals²⁴. In their experimental work, TKO and WT mice (six per group) were exposed to 2 cigarettes twice daily by nose-only exposure or room air for 6 months. It was found that *Mrp1/Mdr1a/1b* knock-out mice had a reduced inflammatory response to cigarette smoke, and lower expression levels of several cytokines and chemokines in lungs independent of smoke exposure. It was suggested that dysfunction of *MDR1* might be one of contributing factors for reduced inflammatory response to inhalation of cigarette smoke and insufficient clearance of toxic substance as a result of its impaired function²⁴.

In rats, *Mdr1a* and *Mdr1b* mRNA expression were found to be highest in the ileum²⁵. The *Mdr1a* expression level in rat lung was 2% of the expression in ileum and expression of *Mdr1b* was 47% of that in ileum. For the purpose of *in vivo* study of P-gp distribution, nude rats were injected with a P-gp overexpressing small cell lung cancer (SCLC) cell line (GLC4/P-gp) and with a P-gp negative cell line (GLC4) (ref.²⁶). P-gp function was visualized with radiolabeled P-gp substrate [11C] verapamil by positron emission tomography (PET) with or without P-gp modulator cyclosporine A. The accumulation of [11C] verapamil was significantly increased by cyclosporine A in brains and GLC4/P-gp tumors in these rats. In all other investigated organs including lungs, the accumulation after cyclosporine A treatment was unaltered.

In intact rabbit lung, vascular P-gp kinetics was measured *in vivo* using the lipophilic amine dye rhodamine 6G (R6G) by measuring R6G in the perfusate during circulation²⁷. Inhibition of P-gp function with verapamil or GF120918 resulted in higher accumulation of R6G in lung. It was proposed that the opposite would happen when epithelial P-gp was inhibited because R6G would then be retained in the airspace. Authors suggested that the inhibition of epithelial P-gp could also result in higher R6G accumulation, with consequent inhibition of R6G transport to the lumen which could be compensated by reversed transport to the interstitial side where it either might be retained in the tissue or transported into the

circulation²⁷. This model could be useful in testing a large variety of pulmonary therapeutic agents, such as corticosteroids and sympathicomimetics that may be substrates for transporters in the lung or modulate their activity.

***In vitro* studies of *MDR1* – respiratory cell lines and tumor cells**

Different approaches have been employed for *in vitro* investigations of P-gp expression and functionality in respiratory cell lines and lung cancer cells. One of cell lines used for drug metabolism studies is the immortalized human bronchial epithelial cell line 16HBE14o-, which resembles primary epithelium²⁸. In these cells, P-gp functional activity was measured with P-gp substrate rhodamine 123 and its transport was inhibited by verapamil²⁹. In research by Hamilton et al., two lung cell lines, Calu-3 (suitable for drug transport studies because of tight junction formation in cell culture) and A549 cells, were compared for P-gp expression and functionality³⁰. P-gp expression was higher in A549 cells than in Calu-3 cells, although the efflux of rhodamine 123 was higher in Calu-3 cells. This may be explained by additional and overlapping activity of other ABC transporters in these cells³¹.

In primary rat alveolar type II cells, *Mdr1b* mRNA levels increased in a time dependent manner in cultures at day 1, 2 and 3 compared to freshly isolated cells. *Mdr1b* mRNA was present at low levels and increased after oxygen radical induction with paraquat³². In freshly isolated primary human bronchial epithelial cells, P-gp was present and increased after 24 hours paraquat exposure. Also, rhodamine 123 efflux was detectable in these cells, which proved functional activity of P-gp (ref.³²), and demonstrated that expression of P-gp is greater during stress, probably as a consequence of radical production and *in vitro* culturing or differentiation.

***MDR1* in respiratory diseases**

The influence of *MDR1* polymorphisms and P-gp protein expression on side effects and clinical responses to important drugs was broadly investigated, especially considering anti-neoplastic agents, antidepressants and immunosuppressant¹. A large number of studies have also focused on the associations between *MDR1* polymorphisms and pathogenesis of various diseases, to mention only few - Parkinson's disease, epilepsy, SLE, inflammatory bowel diseases, cancers and renal disease, rheumatoid arthritis, hypertension⁹ and FMF (Familial Mediterranean fever) (ref.³³). The impact of *MDR1* polymorphisms and level of P-gp protein expression on lung diseases is still speculative, but some authors propose that polymorphisms in the *MDR1* gene may be clinically important particularly in patients with cystic fibrosis, since it was found that one of ABC transporters, a cystic fibrosis transmembrane conductance regulator (CFTR) mutations, are responsible for the onset of that disease³⁴. A certain degree of correlation was also suggested in COPD (ref.³⁵), as well as in lung cancer pathogenesis and treatment.

***MDR1* and COPD**

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality worldwide³⁶. In addition to genetic and other environmental factors, smoking contributes to the development of COPD by overloading of detoxification system and causing the imbalance within the protease-anti-protease system. Results of some studies suggested that P-gp protein plays a role in combating the toxic effect of smoking and in the removal of oxidative stress metabolites^{37,38}. The gene has also been shown to play a role in cellular regeneration³⁹. In the study performed by van der Deen et al., MDR proteins exhibited a protective effect against oxidative stress. It was found that COPD patients had a decreased level of MDR proteins in their bronchial epithelium⁴⁰. Other authors have detected reduced P-gp expression and activity during inflammation⁴¹. In the study by Dogan et al., forty-one participants with COPD were genotyped for *MDR1* 3435C/T polymorphism. When compared to the control group, participants with COPD were significantly more frequently homozygous and heterozygous for *MDR1* C3435T polymorphism³⁵. The T-allele frequency was significantly higher in COPD patients when compared to the healthy control participants (52% and 19%, respectively). These findings suggest that *MDR1* may play a role in the development of COPD through some inflammatory and detoxification mechanisms.

***MDR1* in lung tumors**

Data suggest that P-gp expression in lung cancer is initially small, but changeable, and increasing with disease evolution as a consequence of the acquired resistance to the cancer chemotherapy⁴². Besides, some studies have shown higher P-gp expression at the invasion front of lung tumors and it was suggested that P-gp expression is in a positive correlation with invasion potential of tumor cells⁴³. However, in a study performed by Cordon-Cardo et al., only two out of 22 non-small cells lung carcinoma (NSCLC) samples (both adenocarcinomas) stained positive with three P-gp antibodies and no P-gp was detected on pulmonary carcinoids²¹. The possible effect of *MDR1* polymorphisms was also studied in tobacco-related lung cancer, but no clear association was found between the T/T genotype of the C3435T polymorphism and susceptibility to lung cancer in a group of 268 Caucasian men who were current smokers⁴⁴.

An altered P-gp expression can imply the efficacy of lung cancer chemotherapeutic treatment. Level of P-gp expression might impair the intracellular retention of anticancer drugs including vinca alkaloids, taxanes⁴⁵, cisplatin⁴⁶, and others. The presence of *MDR1* gene polymorphisms could be a cause of this changed expression, especially *MDR1* 3435C and 2677G, which were found to be associated with the lower P-gp expression and consecutive better response to docetaxel chemotherapy⁴⁷. However, these results are not in consent with previous data by Isla et al., in which no relation was found between SNP C3435T in *MDR1* and survival in 62 docetaxel cisplatin treated NSCLC patients⁴⁸. In another research,

a relation between P-gp and glutathione S-transferase pi (GST-pi) expression in NSCLC exposed *in vitro* to doxorubicin was revealed⁴⁹, suggesting that these two factors play a role in doxorubicin resistance. There was also a correlation between current smoking and doxorubicin resistance of NSCLC. Forty-two out of 72 NSCLC smokers expressed P-gp, whereas only two out of 22 tumors of non-smokers were P-gp positive⁵⁰.

In cancer therapy, many attempts have been made to reverse MDR mechanisms. However, in a randomized double-blind trial in 130 SCLC patients no positive effects were seen with the P-gp modulator megestrol acetate in addition to chemotherapeutic drugs, suggesting that levels of P-gp expression in lung tumors were not relevant or that modulation of P-gp activity was not complete in this treatment⁵¹. Although immunosuppressive agents such as cyclosporine A and tacrolimus (both calcineurin antagonists) are P-gp substrates, no relation was found of *MDR1* G2677T and C3435T genotypes with tacrolimus blood levels in 83 lung transplant patients treated with tacrolimus⁵². Altogether, these data implicate that there is still no clear association between *MDR1* polymorphisms and effects on outcome of treatment of lung cancer or lung transplant patients.

In the investigation by Trussardi-Regnier et al., expression of three major resistance genes *MDR1*, *MRP1* and *LRP* was simultaneously investigated in small cell lung cancer, non-small cell lung cancer and metastasis⁵³. Single biopsies of bronchoscopy from 73 patients were performed to investigate expression of these three resistance genes by reverse transcriptase-polymerase chain reaction. Relations between gene expression and patient age, smoking status, histology, and chemotherapy were evaluated. A more frequent expression of *MDR1* (77 versus 66%), *MRP1* (91 versus 72%) and *LRP* (77 versus 63%) genes was detected in the malignant biopsies than in the non-malignant, respectively. In the metastasis biopsies, expression of these genes was markedly increased. Biopsies from progressing cancer showed higher *MDR1*, *MRP1* and *LRP* gene expression. These findings have revealed the high gene expression of *MDR1* and *MRP1* in relapsed diseases⁵³.

CONCLUSION

Although the physiological role of *MDR1* is already well-known and widely investigated, the exact implications of its polymorphisms and P-gp expression in respiratory diseases are still speculative. One of possible reasons for that is the complexity of lung architecture, which makes research on detailed cellular processes very challenging. There are numerous proofs that ABC transporters in general are very highly expressed in the lung. The best evidences so far have been obtained from experimental models, in which the exposure to cigarette smoke in ABC transporter deficient animal models was investigated. The finding of a reduced inflammatory response and lower expression levels of several cytokines and chemokines in lungs could be a suggestive result, leading to the con-

clusion that dysfunction of *MDR1* might be one of contributing factors for reduced inflammatory response to inhalation of cigarette smoke and inadequate clearance of harmful matters as a result of its impaired function. Although scarce, there are also results which indicate that presence of *MDR1* 3435 C/T polymorphism could contribute to the development of COPD in humans. Unfortunately, only a small number of patients were included in investigations so far, insufficient to make a clear conclusion about the clinical relevance of *MDR1* genotyping in COPD patients.

Cell line models have been used to study transport processes and pulmonary drug metabolism. The delivery of pulmonary drugs to the site of action is probably highly dependent on the presence and activity of many ABC transporters, and *MDR1* as one of them, in several cell types in the lung. The first barrier after inhalation is the pulmonary epithelium and transporters in the pulmonary endothelium may be critical for the delivery of intravenously or orally administered drugs. Our present knowledge is not extensive enough for a clear and clinically useful conclusion about the modifications in bioavailability of drugs used for treatment of lung cancer and their efficacy, related to *MDR1* expression. Therefore, the more in-depth research considering the P-gp expression and genotyping of *MDR1* in respiratory diseases is essential, in order to define its role in biology of respiratory diseases, and probably even more important, to personalize the treatment and answer the questions about evolution and prognosis of severe respiratory problems.

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