Expression profiles of vault components MVP, TEP1 and vPARP and their correlation to other multidrug resistance proteins in ovarian cancer

WITOLD SZAFLARSKI1, PATRYCJA SUJKA-KORDOWSKA1, BARTOSZ PULÀ2, KAROLINA JASZCZYŃSKA-NOWINKA3, MALGORZATA ANDRZEJEWSKA1, PIOTR ZAWIERUCHA1,2, PIOTR DZIEGIEL1,2, MICHAŁ NOWICKI1, PAVEL IVANOV5,6 and MACIEJ ZABEL1,2

1Department of Histology and Embryology, Poznan University of Medical Sciences, 60-781 Poznań; 2Department of Histology and Embryology, Wrocław Medical University, 50-368 Wrocław; 3Department of Gynaecological Oncology, Poznan University of Medical Sciences, 60-569 Poznań; 4Department of Anatomy, Poznan University of Medical Sciences, 60-781 Poznań, Poland; 5Division of Rheumatology, Immunology and Allergy, Brigham and Women’s Hospital; 6Department of Medicine, Harvard Medical School, Boston, MA 02115, USA

Received March 7, 2013; Accepted April 26, 2013

DOI: 10.3892/ijo.2013.1975

Abstract. Vaults are cytoplasmic ribonucleoprotein particles composed of three proteins (MVP, TEP1, vPARP) and vault-associated RNAs (vRNAs). Although the cellular functions of vaults remain unclear, vaults are strongly linked to the development of multidrug resistance (MDR), the major obstacle to the efficient treatment of cancers. Available published data suggest that vaults and their components are frequently upregulated in broad variety of multidrug-resistant cancer cell lines and tumors of different histological origin. Here, we provide detailed analysis of vault protein expression in post-surgery ovarian cancer samples from patients that were not exposed to chemotherapy. Our analysis suggests that vault proteins are expressed in the ovaries of healthy individuals but their expression in cancer patients is changed. Specifically, MVP, TEP1 and vPARP mRNA levels are significantly decreased in cancer samples with tendency of lower expression in high-grade tumors. The pattern of vault protein mRNA expression is strongly correlated with the expression of other MDR-associated proteins such as MDRI, MRPI and BCRP. Surprisingly, the protein levels of MVP, TEP1 and vPARP are actually increased in the higher-grade tumors suggesting existence of post-transcriptional regulation of vault component production.

Introduction

Multidrug resistance (MDR) remains to be a major factor in the failure of chemotherapy (1). The mechanisms underlying MDR include the overexpression of transmembrane transporter proteins [P-glycoprotein (Pgp) and multidrug resistance protein (MRP)], which work as drug efflux molecular pumps, or overexpression of DNA repair enzymes and anti-apoptotic molecules to modulate survival of cancer cells after antitumor treatment (2). While various distinct mechanisms of MDR have been identified, these mechanisms are inter-connected to build a complex network of metabolic and signaling pathways that comprise an individual MDR phenotype in a selected population of cancer cells. Deciphering and characterization of such MDR patterns is a main challenge for successful cancer treatment.

Vaults are the largest ribonucleoprotein particles reported to date. They were first observed as small oval ‘contaminants’ of clathrin-coated vesicles in a rat liver homogenate fraction (3). Subsequently, they were isolated and characterized from a broad spectrum of lower and higher eukaryotes (reviewed in ref. 4). The high abundance of vaults in eukaryotic organisms suggests their basic, yet not well characterized, function in cellular metabolism (5). Vaults are especially abundant in the cytoplasm of epithelial cells of a digestive tract, bronchial cells and macrophages (6) that might be indicative for their function in immunity and/or phagocytosis.

Vaults are evolutionary conserved particles with hollow barrel-like structure (3). Their main structural component is a 110 kDa major vault protein (MVP, Swiss-Prot no. Q14764), which is able to assemble vault particle spontaneously (7). In addition to MVP, two other minor vault proteins exist: the 193 kDa vault poly (ADP-ribose) polymerase (vPARP)
and the 240 kDa telomerase-associated protein-1 (TEP-1). TEP-1 directly interacts with another vault constitutive, small non-coding vault RNAs (vRNAs). While the structural role of MVP in vault assembly is well-documented, minor vault proteins and vRNAs are probably involved only in the stabilization of vault particle. In addition, TEP1 and vPARP might associate with the vaults only transiently.

Initial biochemical data demonstrated the presence of 96 MVP, 8 vPARP and 2 TEP-1 as well as at least 6 vRNA copies in the vault molecule (8). Recent high-resolution structure revealed that the single vault particle is comprised of two half-vaults. These half-vaults consist exclusively of 39 MVP proteins lacking minor vault components (9), although they can still reside within vault shell-like structures in vivo conditions. Interestingly, vaults are very dynamic molecules that can open their shell-like structures to dissociate their halves, which in turn can also be exchanged between different vault molecules.

Despite our knowledge of vault composition and structure, functions of vaults and their components are mostly unknown. While in vitro studies proposed that vaults and MVP participate in signal transduction and nucleocytoplasmic transport, genetic studies with MVP knockout mice (which lack vaults) did not reveal any phenotype under normal conditions. In 1995, Scheffer et al demonstrated that MVP protein is a homologue of lung resistance-related protein (LRP), factor responsible for development of multidrug resistance (MDR) in cancer cells (10). Indeed, MVP is overexpressed in several MDR tumor cell lines thus making MVP and/or vaults possible biomarkers for prediction of chemotherapy success.

An analysis of 61 cell lines from NCI panel representing a variety of human cancer types revealed that LRP protein was overexpressed in 78% of the cell lines and its higher expression generally correlates with the MDR phenotype (11). These results were confirmed on the other 8 cell lines demonstrating higher LRP expression in the drug-resistant cells (12). The overexpression of MVP observed in the astrocytoma cell line also correlated with resistance against broad spectrum of drugs such as doxorubicin, methotrexate, etoposide, vincristine, cytarabin and cisplatin (13). The activity of vaults and MVP seems to be tissue-specific; in non-small cell lung cancer the higher expression of MVP correlated only with resistance to cisplatin treatment but not against daunorubicin, doxorubicin, etoposide, vincristine, vinblastine and bleomycin (14).

The role of MVP/vaults for the MDR development in ovarian cancer is not well characterized. Here, we extended our initial studies on the profiling of vault proteins MVP, TEP1 and vPARP in ovarian cancer patients that have not undergone chemotherapy (15). We show that MVP, TEP1 and vPARP mRNA levels are significantly decreased in cancer samples compared to non-cancerous ones. Moreover, higher-grade tumors demonstrate lower MVP, TEP1 and vPARP mRNA levels. The pattern of vault proteins mRNA expression is strongly correlated with expression of other MDR-associated proteins such as MDR1, MR1 and BCRP. Surprisingly, the protein levels of MVP, TEP1 and vPARP are actually increased in the higher-grade tumors suggesting existence of post-transcriptional regulation of vault component production.

Materials and methods

Clinical material. A total of 50 ovarian post-surgery specimens were provided by Department of Gynaecological Oncology, Poznań University of Medical Sciences. Thirty-seven specimens were analyzed using RT-qPCR, 27 were analyzed using IHC and 12 were analyzed using both, RT-qPCR and IHC. Approval for the study was given by the Ethics Committee of Poznań University of Medical Sciences. We used specimens only from patients who had not received any treatment before surgery. All details including diagnosis and histopathological parameters for patients taken into research and techniques applied for analysis are presented in Table I.

Isolation of total RNA and synthesis of cDNA. The post-surgery tissue materials were stored in RNA Stabilization Solution (RNAlater® , Applied Biosystems) at -80°C. The total RNA fraction was prepared using TRI Reagent® Solution (Applied Biosystems) and then purified on GeneMatrix Universal RNA Purification Kit (EURx). Total RNA was determined by measuring optical density at 260 nm and purity was estimated by 260/280 nm absorption ratio, which was consistently >1.8 (NanoDrop® ND-1000, Thermo Scientific). RNA integrity was assessed by electrophoresis in 1% agarose gel with ethidium bromide. All RNA samples were stored in H₂O at -80°C until used.

Reverse transcription was carried out on 1.0 µg of total RNA following the manufacturer’s protocol (First Strand cDNA Synthesis Kit, Fermentas). Template RNA, random hexamer primers (1 µl) and DEPC-treated water were mixed together to a total volume of 11 µl and pre-incubated at 65°C for 5 min in the Biometra Thermocycler. Then the sample was chilled on ice, centrifuged to spin it down and moved to a fresh PCR tube in order to prevent the permeability of the cup. Subsequently, 5X reaction buffer (4 µl), RiboLock™ RNase inhibitor 20 U/µl (1 µl), 10 mM dNTP mix (2 µl) and M-MuLV reverse transcriptase 20 U/µl (2 µl) were added to the pre-incubated solution, mixed by pipetting and incubated at 42°C for 60 min (Biometra Thermocycler). Finally, cDNA was stored in H₂O at -20°C or immediately used for subsequent amplification reactions.

Absolute quantification of mRNA copies - preparation of standard curves for a copy number determination. cDNAs of individual genes were amplified using standard PCR. Primers were used as indicated in Table II. PCR products were analyzed in 2% agarose gel in order to confirm their specificity and then all PCR products were purified separately by GeneMatrix PCR/DNA Clean-Up Purification Kit (EURx). The concentration of each DNA was estimated by measuring optical density at 260 nm (NanoDrop ND-1000). The weight concentrations were converted to the corresponding DNA copy number using Avogadro constant:

\[
\text{DNA (copy)} = \frac{6.02 \times 10^{23} \text{ (copies mol}^{-1}\text{)} \times \text{DNA amount (g)}}{\text{DNA length (bp) x 660 (gmol}^{-1}\text{ bp}^{-1})}
\]

A 10-fold serial dilution of corresponding DNA and specific primers, ranging from 1x10⁷ to 10 copies per µl, were
used to construct the standard curves. Threshold cycle (C<sub>t</sub>) values in each dilution were measured in duplicate and were plotted against the logarithm of their initial template copy numbers. Each standard curve was generated by a linear regression of the plotted points. From the slope of each curve, PCR amplification efficiency (E) was calculated according to the following equation: $E = 10^{\frac{-1}{\text{slope}}}-1$.

**Real-time PCR reaction.** Each cDNA (1 µl) was added to the reaction mixture composed of 12.5 µl 2X Maxima® SYBR-Green/ROX qPCR Master mix (Fermentas), 1 µl specific pair of primer (f.c. 0.3 µM) and 10.5 µl H<sub>2</sub>O. The reactions were driven in twin.tec real-time PCR plates with PCR Film (Eppendorf) using Mastercycler ep realplex<sup>2</sup> (Eppendorf). The PCR program was as followed: i) initial denaturation, 95°C, 10 min; ii) denaturation, 95°C, 15 sec; iii) annealing 60°C, 30 sec; and iv) extension 72°C, 30 sec. The number of cycles was 40-50. Melting curves were made and 2% agarose gel electrophoresis was used to verify the amplification product specificity and size, respectively. All samples were amplified in duplicate or triplicate and in case that results varied more than 15% the reactions were repeated.

**Absolute quantification by real-time PCR.** Absolute quantification method was used to quantify the MVP, TEP1 and vPARP mRNA copy number. Absolute quantification determines the exact copy concentration of a target gene by relating the C<sub>t</sub> value to a standard curve. Prior to absolute quantification the C<sub>t</sub> values were normalized by comparison to the C<sub>t</sub> average obtained from five housekeeping genes (β-actin, B2M, GAPDH, HPRT1, MRLP19; for details see Table II). Primers for studies on expression of vault-related gene and reference genes (Table II) were purchased from the Laboratory of DNA Sequencing and Oligonucleotide Synthesis, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw.

**Immunohistochemistry.** Immunohistochemistry was performed on transverse 5 µm formalin-fixed paraffin-embedded sections from human ovarian carcinoma. The slides were de-waxed with xylene, and gradually hydrated. Activity of endogenous peroxidase was blocked by 30 min exposure to 1% H<sub>2</sub>O<sub>2</sub>. Monoclonal mouse anti-human MVP (LRP-56, 1:20 dilution, Abcam), polyclonal rabbit anti-human TEP1 (P193-10, 1:40 dilution, Abcam) and monoclonal mouse anti-human PARP4 (P193-10, 1:40 dilution, Abcam) were used as the primary antibodies. Tested sections were incubated with primary antibodies overnight at 4°C, followed by incubation with EnVision Detection System Peroxidase/DAB, Rabbit/Mouse (Dako) for 30 min. The sections were then finally reacted with 3,3-diaminobenzidine, counterstained with hematoxylin, dehydrated and mounted. The protein expression was determined and described using Remmele scale (IRS) (16) with modifications (17).

**Statistical analysis.** Spearman’s rank correlation was applied to examine the associations between variables. For multiple
unpaired samples (tumor and cancer) Mann-Whitney test was used. Differences and correlations were assumed to be statistically significant at the level of p<0.05. Statistical analysis was performed using Statistica PL software (ver. 10).

Results

Gene expression of vault components MVP, TEP1 and vPARP, and their relation to expression of other MDR-associated genes and ovarian cancer marker genes in control and cancer specimens. To analyze whether vault proteins demonstrate coordinated expression within vault-associated group (MVP, TEP1 and vPARP) and its possible correlation to the expression of the other MDR-associated genes, we performed quantification of mRNA levels in cancerous and control samples from patient ovaries (37 post-surgical samples). Levels of mRNAs encoding vault components were compared to four genes known to be involved in MDR mechanisms in cancer [MDR1 (encodes glycoprotein P, ABCB1), MRPI (ABCC1), MRP2 (ABCC2) and BCRP (ABCG2)] (18). Three genes (MUC1, CA125 and CLDN3), which are validated ovarian cancer markers (19), were monitored to confirm cancerous nature of our study post-surgical specimens.

Indeed, we found that expression of MUC1 and CLDN3 is significantly higher in cancer patient samples than in control samples. We also observed higher expression of CA125, although without statistical significance in our group of patients (Fig. 1). The mRNA levels of MVP, TEP1 and vPARP were significantly decreased in cancer samples when compared to the normal ovary (Fig. 1). From the other MDR-associated genes, MDR1 mRNA levels were decreased while levels of MRP1/MRP2 mRNAs were increased without reaching statistical significance, and BCRP expression remains similar in both groups of patients.

Correlation of gene expression between gene-encoded vault particles, MDR-associated genes and cancer marker proteins. The gene expression results obtained by quantification of the mRNA levels were used to perform non-parametric Spearman array correlations (Table III). First, calculations

Table II. Sequences of RT-QPCR primers used in the studies and expected PCR product lengths.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>NCBI accession number</th>
<th>Product length (bp)</th>
<th>Sequence</th>
</tr>
</thead>
</table>
| MVP       | NM_017458             | 135                 | F: 5'-TGAGGAGGTTCTGGATTTGG  
R: 5'-TGCACTGTATTACACGCACCTC |
| TEP1      | NM_007110             | 186                 | F: 5'-GCTCAAGAGGAGAAGCTGA  
R: 5'-GGACAGAGCCTGCTGCTATT |
| vPARP     | NM_006437             | 207                 | F: 5'-GCCAAATACCGAGCTTTGAG  
R: 5'-AGGAGAACCATGCAACAAAG |
| MDR1      | NM_000927             | 131                 | F: 5'-TGACAGCTACAGACGAGAAAG  
R: 5'-TCTTCACTCCAGGGTCCTGAGT |
| MRPI      | NM_004996             | 149                 | F: 5'-TCTGATCTAGATGTGCTGGAG  
R: 5'-TGAGGAGGAGAGAGCAGACTAA |
| MRP2      | NM_000392             | 115                 | F: 5'-CTCGGAATGTGAATAGCTG  
R: 5'-TTGCTTGTAGGTACTCCAGG |
| BCRP      | NM_004827             | 128                 | F: 5'-TTCGGTTGAGCAACAACCTATG  
R: 5'-TCCAGACACCCACAGGATAA |
| MUC1      | NM_002456             | 128                 | F: 5'-TGAGGAGGAGGAGAAGCTGA  
R: 5'-CCACTGCTGGGTGTTTGA |
| CA125     | NM_024690             | 215                 | F: 5'-ACAAGGAGCAAGAGAAGACTAA  
R: 5'-GGTTGACTCAGAGGAGACAG |
| CLDN3     | NM_001306             | 246                 | F: 5'-AAGGGTGTAAGACTGCTGCT  
R: 5'-AGTCCCGGATATGTGTGTTG |
| β-ACT     | NM_001101             | 169                 | F: 5'-TCTGAGCACCACACACCTTAC  
R: 5'-GATAGCAGCCAGGGATCTCC |
| B2M       | NM_004048             | 137                 | F: 5'-CTCACAGTCATCCAGCAGAGA  
R: 5'-AAGACAAGTCTAGATGGTCC |
| GAPDH     | NM_002046             | 199                 | F: 5'-GAAAGGTTGAGGCTGGAGTTCA  
R: 5'-CTTGAGAAGCGGAAAGCTTGGT |
| HPRT1     | NM_000194             | 156                 | F: 5'-CTGAGGATTGGGAGGGAGTTG  
R: 5'-AACTTCAGGGAGCTACAGAAG |
| MRPL19    | NM_014763             | 171                 | F: 5'-ACTTTAATAATCTCAGGGTC  
R: 5'-ACTTTACGCTCAATACAG |

unpaired samples (tumor and cancer) Mann-Whitney test was used. Differences and correlations were assumed to be statistically significant at the level of p<0.05. Statistical analysis was performed using Statistica PL software (ver. 10).

Results

Gene expression of vault components MVP, TEP1 and vPARP, and their relation to expression of other MDR-associated genes and ovarian cancer marker genes in control and cancer specimens. To analyze whether vault proteins demonstrate coordinated expression within vault-associated group (MVP, TEP1 and vPARP) and its possible correlation to the expression of the other MDR-associated genes, we performed quantification of mRNA levels in cancerous and control samples from patient ovaries (37 post-surgical samples). Levels of mRNAs encoding vault components were compared to four genes known to be involved in MDR mechanisms in cancer [MDR1 (encodes glycoprotein P, ABCB1), MRPI (ABCC1), MRP2 (ABCC2) and BCRP (ABCG2)] (18). Three genes (MUC1, CA125 and CLDN3), which are validated ovarian cancer markers (19), were monitored to confirm cancerous nature of our study post-surgical specimens.

Indeed, we found that expression of MUC1 and CLDN3 is significantly higher in cancer patient samples than in control samples. We also observed higher expression of CA125, although without statistical significance in our group of patients (Fig. 1). The mRNA levels of MVP, TEP1 and vPARP were significantly decreased in cancer samples when compared to the normal ovary (Fig. 1). From the other MDR-associated genes, MDR1 mRNA levels were decreased while levels of MRP1/MRP2 mRNAs were increased without reaching statistical significance, and BCRP expression remains similar in both groups of patients.

Correlation of gene expression between gene-encoded vault particles, MDR-associated genes and cancer marker proteins. The gene expression results obtained by quantification of the mRNA levels were used to perform non-parametric Spearman array correlations (Table III). First, calculations
Table III. Correlations between expression of examined genes.

<table>
<thead>
<tr>
<th></th>
<th>MVP</th>
<th>TEP1</th>
<th>vPARP</th>
<th>MDR1</th>
<th>MRP1</th>
<th>MRP2</th>
<th>BCRP</th>
<th>MUC1</th>
<th>CA125</th>
<th>CLD3</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVP</td>
<td>1.000</td>
<td>0.813</td>
<td>0.680</td>
<td>0.480</td>
<td>0.595</td>
<td>0.691</td>
<td>0.224</td>
<td>0.029</td>
<td>0.029</td>
<td></td>
</tr>
<tr>
<td>TEP1</td>
<td>1.000</td>
<td>0.622</td>
<td>0.536</td>
<td>0.532</td>
<td>0.104</td>
<td>0.627</td>
<td>0.045</td>
<td>0.176</td>
<td>0.121</td>
<td></td>
</tr>
<tr>
<td>vPARP</td>
<td>1.000</td>
<td>0.407</td>
<td>0.497</td>
<td>0.104</td>
<td>0.104</td>
<td>0.662</td>
<td>0.003</td>
<td>0.262</td>
<td>0.049</td>
<td></td>
</tr>
<tr>
<td>MDR1</td>
<td>1.000</td>
<td>0.259</td>
<td>0.267</td>
<td>0.018</td>
<td>0.040</td>
<td>0.294</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRP1</td>
<td>1.000</td>
<td>0.390</td>
<td>0.529</td>
<td>0.513</td>
<td>0.502</td>
<td>0.349</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRP2</td>
<td>1.000</td>
<td>0.338</td>
<td>0.292</td>
<td>0.377</td>
<td>0.055</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCRP</td>
<td>1.000</td>
<td>0.026</td>
<td>0.218</td>
<td>0.186</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUC1</td>
<td>1.000</td>
<td>0.591</td>
<td>0.790</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA125</td>
<td>1.000</td>
<td>0.554</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLD3</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Significant r-values are given in bold (n=35).

Figure 1. Analysis of the transcript expression of vault components in control and cancerous tissues of the ovary in the patient specimens. Normal ovary (n=5), cancer (n=17). Significant changes determined by Mann-Whitney test, i.e. MVP (p=0.0033), TEP1 (p=0.01359), vPARP (p=0.0054), MUC1 (p=0.0042) and CLDN3 (p=0.0054).
revealed significant correlations (p<0.05) between genes encoding vault proteins MVP, TEP1 and vPARP. Second, highly significant correlations were observed within the group representing MDR-associated genes (MVP, TEP1, vPARP, MDR1, MRP1, BCRP) with exception of MRP2. Finally, cancer marker genes (MUC1, CA125, CLDN3) strongly correlate with each other (0.591, 0.79, 0.554) and with MRP2 expression.

**Immunohistochemical (IHC) analysis of the vault components in ovarian cancer.** A total of 26 samples from post-surgical specimens were analyzed using immunohistochemistry with selected antibodies. The protein expression levels of MVP, TEP1 and vPARP were determined according to the Remmele Scoring System (Fig. 2). Levels of MVP had a wide range of expression in the analyzed specimens (0-12) with the average equal to 5.3 (n=26). Similarly, vPARP showed wide expression profile (0-9) with the average equal to 4.9 (n=26). The lowest overall expression was demonstrated in TEP1 (average 3.3 and the range 0-8, n=26).

**Expression of vault components in different grading of ovarian cancer.** Two independent techniques (IHC and RT-qPCR) were applied in order to demonstrate different expression of MVP, TEP1 and vPARP in the grading stages of ovarian cancer. Interestingly, the level of mRNA for all three analyzed genes was decreased (Fig. 3) in the range of higher grading, however, their protein products (MVP, TEP1 and vPARP) increased. For example, average expression of mRNA for vPARP decreased from 7421 mRNA copies per 1 µg of total RNA in patients with G1 grading (n=7) to 2887 mRNA copies per 1 µg of total RNA in patients with G2 or G3 grading (n=22). However, vPARP protein expression increased from 1.25 (n=4) to 5.13 (n=16) in G1 and G2/G3 samples, respectively.

**Discussion**

MDR is the major obstacle in our attempts to improve clinical outcome in ovarian carcinoma patients (1). The main mechanisms of tumor cell resistance to chemotherapeutic
drugs are associated with activation of transporter proteins that increase drug efflux from cancer cells, or with activation of anti-apoptotic mechanisms (2). Despite many years of research these anti-drug resistance mechanisms are poorly understood. One of the reasons is the intrinsic heterogeneity of many cancers including ovarian cancer. This heterogeneity warrants further investigation of gene expression profiles that will classify subgroups of tumors according to their gene expression patterns. Such classification will allow ‘personalized’ approach in the choice of chemotherapy treatment for the individual patient.

Here, we characterized expression profiles of vault particle proteins MVP, TEP-1 and vPARP, the candidate molecules that might be involved in both MDR and development of ovarian cancer. Vaults are found frequently in cancer cell lines and tissues (20). While vaults and their components are implicated in MDR for many years, the mode of vault/vault component-mediated MDR is not clear. They are thought to be involved in the nuclear elimination of chemotherapeutic drugs by unknown mechanism (21). Interestingly, the range of drugs eliminated by vaults or their components is even broader than those associated with classical P-glycoprotein-associated MDR and includes alkylating agents such as melphalan and cyclophosphamide and platinum compounds (6).

The current study attempted to determine the gene expression patterns of vault components, other MDR-associated proteins and marker proteins in ovarian cancer. While earlier data clearly demonstrated that higher MVP synthesis in the cancer cells caused MDR phenotype (22) and higher expression levels of MVP correlate with lower survival rate of patients (23), the rest of vaults components were not analyzed in the same settings. Furthermore, ectopic overexpression of the MVP alone is not sufficient to confer a drug resistant phenotype (10). This is not surprising because MVP might require coordinated synthesis of other vault particle components such as minor vault proteins and vRNA for the development of MDR.

We found that levels of all three protein components of vaults are decreased on mRNA abundance level in cancer tissues. Decrease in the expression of vault protein genes on transcriptional/mRNA abundance levels is well correlated with decrease of MDR1 mRNA levels and with general increase of expression levels of other MDR-associated genes as well as cancer marker genes (MUC1, CA125, CLDN3) in ovarian cancer patients. Since expression of MVP and MDR1 is known to be upregulated in response to chemotherapeutic drugs (11,13), this might suggest that vault proteins and MDR1 are also co-induced by drug treatment. Whether such co-induction exists remains to be determined.

To our surprise, the actual protein levels of MVP, TEP1 and vPARP are significantly increased in cancer ovaries as judged by immunohistochemistry. Moreover, while the protein levels of vault proteins are actually increasing in higher-grade tumors, their mRNA levels are decreasing. The observed discrepancy can be explained by the regulation on the post-transcriptional level, i.e. by more efficient translation of mRNAs encoding vault components in tumors than in normal tissues. It also can be that vault proteins are more stable in higher-grade cancers due to the more efficient incorporation into vault particles, difference in the subcellular localization [such as docking into the nuclear pore complexes (24)] or the presence/absence of specific post-translational modifications. Future studies are required to answer these questions.
Acknowledgements

This study was supported by the Polish Ministry of Science and Higher Education (grant no. 0172/B/P01/2009/36).

References


