Original Article

MTAP deficiency is associated with an unfavourable prognosis and platinum resistance in ovarian cancer

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Abstract: Methylthioadenosine phosphorylase (MTAP), a key enzyme of adenosine salvage pathway, has been reported to be high frequency absence or reduced in various human tumors. This study was to investigate the expression of MTAP in ovarian cancer (OC) and the association with platinum based chemotherapy response and prognosis. We first examined the expression of MTAP in OC cell lines and 20 OC tissues. Then we retrieved the expression of MTAP in 90 platinum-resistant and 197 platinum-sensitive ovarian cancers, according to The Cancer Genome Atlas (TCGA) Ovarian Statistics data. Further, the association between MTAP and drug resistance in OC was analyzed by bioinformatic analysis. Last, Kaplan-Meier plotter (KM plotter), was invited to validate the MTAP’s prognostic value in OC, Kaplan-Meier survival plot and P value were calculated. Compared with the parental OC cell lines, low expression of MTAP was found in platinum-resistant cell lines: A2780/DDP (P=0.021), A2780/CBP (P=0.002) and SKOV3/DDP (P=0.008). Similarly, MTAP’s down-expression was also found between 10 platinum-resistant and 10 platinum-sensitive tissues (P=0.003). Running data from TCGA, MTAP in platinum-resistant group was down expressed compared with platinum-sensitive group (P=0.024). Bioinformatic process showed close relationship between MTAP and drug resistance. Computed by KM plotter, low expression of MTAP was associated with OC patients’ poor progression-free survival (P=0.027) and overall survival (P=0.0033). These data demonstrate that low expression of MTAP is associated with platinum based chemotherapy resistance and worse prognosis in OC. Taken together these findings suggest that MTAP could serve as a potential biomarker for prognosis and predictor for platinum based chemotherapy response in OC.

Keywords: Ovarian cancer, methylthioadenosine phosphorylase, prognosis, platinum resistance, biomarker

Introduction

Ovarian cancer (OC) is often referred to as the “The Silent Killer”, the patients were always first diagnosed at an advanced stage [International Federation of Gynecology and Obstetrics (FIGO) stage III/IV], and often progressed rapidly, which results in OC mortality among the top of the gynecology malignant tumors. Standardized surgery supplemented by platinum based chemotherapy is the main way of treatment, about 75% of the early cured people can get the complete response, however, months later, the majority of patients appear tumor recurrence or metastasis, and due to the existence of multiple drug resistance (MDR) of chemotherapy, the 5-year survival rate is only 30-45% of patients with OC [1, 2]. Predictive markers used to guide treatment decisions and prognostic markers to estimate patient outcomes are desperately needed.

Methylthioadenosine phosphorylase (MTAP) is a key enzyme of adenosine salvage pathway, catalyzes 5'-deoxy-5'-methylthioadenosine (MTA) to generate ATP, dAMP and methionine for participating in the energy generation and protein synthesis in the cell. The MTAP is located on chromosome 9p21-22, which is a heritable fragile site. Loss of heterozygosity and deletion of this region usually causes co-deletion of MTAP and some tumor suppressor genes such as p16 in various kinds of tumors. In normal cells and tissues, MTAP, is abundant and acted as a housekeeping gene, but in a variety of malignant tumor cell lines and cancer tissues, it appears high frequency absent or reduced. MTAP deficiency is found in hematologic malig-
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Figure 1. Expression change comparing resistant and sensitive cell lines. The expression of MTAP was significantly reduced in resistant cell lines (*P<0.05, **P<0.01).

nancies [3-6] and solid tumors, such as bladder cancer [7], breast cancer [8], nasopharyngeal carcinoma [9], myxofibrosarcomas [10], non-small cell lung cancer (NSCLC) [11], laryngeal carcinoma [12], etc. In addition, He et al. [9] found down regulation of MTAP had been shown to correlate with advanced tumor stage and poor prognosis in nasopharyngeal carcinoma. In myxofibrosarcomas, MTAP deficiency was associated with an unfavorable prognosis and when MTAP re-expressed in MTAP-deficient cells, the cells’ ability of migration and invasion, proliferation, and anchorage-independent colony formation was inhibited [10].

MTAP mainly functioned as a tumor suppressor in most carcinomas, but in some other malignancies, such as prostate cancer [13] and pilocytic astrocytomas [14], it was up regulated. Information on MTAP-expression in OC is lacking, and its association with chemoresistance and survival has never been systematically studied. In this study, we detected the expression of MTAP and its relationship with OC clinicopathological parameters, trying to clarify its prognostic significance and correlation to platinum resistance.

Materials and methods

Cell culture and tissue samples

The human ovarian cancer cell lines SKOV3 and A2780 were maintained in our lab, and the resistant cell lines A2780/DDP and A2780/CBP cell lines were established from A2780 by exposed to increasing concentrations of cisplatin and carboplatin, respectively. A stable cisplatin-resistant cell line, SKOV3/DDP, was established from SKOV3 by exposure to increasing concentrations of cisplatin continuously [15]. The SKOV3 and A2780 cell lines and their derivative cell lines were routinely maintained in DMEM or RPMI-1640 (CORNING, USA) supplemented with 10% fetal bovine serum (FBS, CORNING, USA), 100 U/ml penicillin, and 100 μg/ml streptomycin (Gibco BRL, Grand Island, NY) at 37°C in a humidified atmosphere containing 5% CO₂.

Ovarian cancer tissues were collected from patients who underwent optimal cytoreductive surgery for primary epithelial ovarian cancer at the department of gynecologic oncology, the Affiliated Tumor Hospital of Guangxi Medical University. All cancer tissues of FIGO stage IB-IV were proved as epithelial ovarian carcinoma by pathology. All patients treated with 6-8 cycles of postoperative platinum based chemotherapy: paclitaxel plus cisplatin (TP) or paclitaxel plus carboplatin (TC). The follow-up of the patients was at least 1 year after completing the chemotherapy. In addition, the patients were assigned into two groups: platinum resistant group (n=10) and the platinum sensitive group (n=10) according to the 2013 guidelines National Comprehensive Cancer Network (NCCN). The median age of the patients was 47 years (range, 28-60 years). The study was endorsed by the Ethics Committee of Affiliated Tumor Hospital of Guangxi Medical University. All patients received an explanation concerning the aims of the study and provided signed informed consent. After surgical removal from the primary lesions, the tissues were frozen in liquid nitrogen tank until they were used for mRNA isolation.

qRT-PCR analysis

Total RNA of ovarian cancer cells/tissues was isolated by TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s protocols and then 1 μg RNA was reverse transcribed using the RevertAid First Strand cDNA Synthesis Kit (Thermo, Austin, TX, USA) to generate cDNA. Quantitative real-time polymerase chain reaction (qRT-PCR) was performed using a standard SYBR® Premix Ex Taq™ II (Takara, Japan). The primers were synthesized by Beijing Genomics Institute (Beijing, China) as follows: MTAP forward primer, 5’-CAGGCGAACAT-
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Reduced expression of MTAP was related to platinum-resistance

By qRT-PCR, we detected the expression difference in ovarian cancer cell lines, the results showed that MTAP was down regulated in platinum-resistant cell lines: A2780/DDP ($P=0.021$), A2780/CBP ($P=0.002$) and SKOV3/DDP ($P=0.008$), in contrast to their parental cell lines A2780 and SKOV3, respectively, as shown in Figure 1. Then we examined the expression difference of MTAP between platinum-resistant and platinum-sensitive epithelial ovarian cancer tissues, the expression of MTAP in platinum-resistant group (7/10) was significantly lower compared with platinum-sensitive group (3/10) ($P=0.003$). To further verify the result, we run the data extracted from TCGA database on the expression of MTAP between platinum-resistant and platinum-sensitive tissues of OC patients. The results showed that expression of MTAP in platinum-resistant group was reduced compared with platinum-sensitive group, the $P$ value was $0.024$, as shown in Figure 2B.

Bioinformatics analysis

Then we searched The Cancer Genome Atlas (TCGA, http://cancergenome.nih.gov) database to get the expression data of MTAP and divided it into two groups according to the clinical chemotherapy response information: platinum-resistant group (n=90) and platinum-sensitive group (n=197). The gene/protein-gene/protein interaction network was generated by GeneMANIA (http://genemania.org/); the Coremine Medical online tool (http://www.coremine.com/medical/) was used to annotate the biological processes. To analyze the prognostic value of MTAP in ovarian cancer, the central server of Kaplan-Meier plotter (KM plotter) for ovarian cancer (www.kmplot.com/ovar) was used to calculate and plot Kaplan-Meier survival plots to assess the correlation between survival and gene expression. We chose the JetSet [16] best probe in the probe set, as it was MTAP (Affymetrix ID: 231984_at), the hazard ratio (HR) and logrank $P$ value were calculated. In the survival analysis, the median were used as the cutoff value and the significance threshold was set at $P<0.05$ [17].

Results

Reduced expression of MTAP was related to platinum-resistance

Figure 2. Expression differences of MTAP between platinum-resistant and platinum-sensitive tissues in ovarian cancer. The expression of MTAP was significantly reduced in chemo-resistant tissues. A. Was the data from 20 ovarian cancer tissues collected by us; B. Represented the data from TCGA database (*$P<0.05$, **$P<0.01$).
Prediction and analysis of function based on gene/protein-gene/protein interactions

To further demonstrate the function of MTAP, we collected the drug-resistance related genes in ovarian cancer using the search terms “Ovarian Neoplasms”, “Drug Resistance”, and “Drug Resistance and Ovarian Neoplasm” from the PubMed online database (https://www.ncbi.nlm.nih.gov/pubmed), and the interaction networks between MTAP and drug resistance-related genes in ovarian cancer were analyzed using the GeneMANIA tool. The results revealed that MTAP was interacted with 21 genes in total (Figure 3), and 12 of them had been verified in reports to have correlation with drug-resistance in ovarian cancer: APC, WNT signaling pathway regulator (APC), mitogen-activated protein kinase1 (MAPK1), nuclear factor kappa B subunit 1 (NFKB1), PLAG1 like zinc finger 1 (PLAGL1), URI1, prefoldin like chaperone (URI1), ATM serine/threonine kinase (ATM), ATR serine/threonine kinase (ATR), Cyclin-dependent kinase inhibitor 2A gene (CDKN2A), check point kinase 2 (CHEK2), death associated protein kinase 1 (DAPK1), F-box protein32 (FBXO32) and phosphatase and tensin homolog (PTEN). Such as CDKN2A, whose down-regulation in ovarian cancers represented an unfavorable prognosis, and had an underlying interaction in paclitaxel resistance in cancer therapy [18,19]. The presence of a germ-line or somatic mutation in CHEK2 gene, one of the homologous recombination genes, was strongly associated with primary platinum sensitivity [20]. In additional, MTAP was also interacting with a long non-coding RNA RP11-145E5.5, which hasn’t been reported before.

**Functional prediction based on the annotated biological process**

Coremine Medical, an online tool, which can help to understand the terms relevant to biological function and importance in disease due to the cross-referenced gene and protein names. Therefore, we used the Coremine Medical and set the probabilistic value lower than 0.009 to annotate MTAP with respect to biological processes. As shown in Figure 4, 23 biological processes closely associated with ovarian cancer, drug resistance, and the MTAP was annotated, suggesting its mutual involvement in drug resistance in ovarian cancer. Among the 23 processes, four (growth, cell growth, cell proliferation and cell division) were cell-growth related, three (metabolic process, polyamine biosynthetic, biosynthetic process) were cell-metabolism related, three (cell cycle, regulation of cell cycle and S phase) were cell-cycle related, and three (methylation, DNA methylation and demethylation) were cell-methylation related. Among these, the correlation of the MTAP’s metabolic function and drug resistance is in accordance with the previous study in which MTAP was shown to affect cell metabolism regulation in cancer therapy [21].

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**Figure 3.** The gene/protein interaction networks of MTAP based on the GeneMANIA online tool.
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Reduced expression of MTAP was related to worse prognosis

Based on the data obtained from KM plotter, we proceeded to investigate the relationship between MTAP expression and clinical outcome. We identified 1,648 patients in GEO (12 independent datasets) and TCGA. In these patients, 1,364 patients get a platinum-based chemotherapy and 780 received paclitaxel, besides, 763 patients were treated with both platinum and paclitaxel. Then we used Jetset to filter for probe set quality and include only reliable and specific probe sets in the statistical evaluation. In all the patients no matter what chemotherapy they were given from 13 independent datasets, down-regulated MTAP was significantly associated with patients’ worse progression-free survival (PFS, HR=0.79, \( P=0.027 \)) and overall survival (OS, HR=0.72, \( P=0.0033 \)), as shown in Figure 5. Then we compared the prognosis of patients treated with different chemotherapy, in the patients treated based on platinum, MTAP’s down-regulation was correlated to OS (HR=0.68, \( P=0.0045 \)), and marginally with PFS (HR=0.81, \( P=0.051 \)). When running data on the patients treated with platinum and paclitaxel, only OS was significant (HR=0.69, \( P=0.02 \)), no apparent significance was found in PFS (\( P=0.26 \)). In the patients treated based on paclitaxel, lower expression of MTAP significantly correlated with OS (HR=0.68, \( P=0.017 \)), but had no significance with PFS (\( P=0.24 \)), as shown in Figure 6.

Discussion

OC is the fifth most common cause of cancer-related death in women. Chemotherapy based on platinum compound and taxane after cytoreductive surgery is the standard treatment in clinic. The platinum-based agent cisplatin has made a great contribution in cancer therapy, especially in testicular and ovarian cancer, since its inception over 40 years ago [22]. However, multiple drug resistance is the main cause of the failure of the ovarian cancer chemotherapy at present stage. Mostly, ovarian cancer patients get chemoresistance after standard treatment, which reduces the treatment outcome and the 5-year survival rate. In general, the potential mechanisms of chemoresistance are decreased drug uptake, disordered DNA damage repair system, obstructed apoptosis pathway or activated cell survival pathway [23]. This study analyzed the association between MTAP level and platinum based chemotherapy response as well as prognosis in OC by using public databases and basic experiments.

For ovarian cancer, to validate a prognostic biomarker is a major challenge in large independent patient cohorts. TCGA database conclude thousands of ovarian cancer microarray datasets released to the public. The data have been reported to be used in analyzing the relationship between the data and the chemotherapy response, the prognosis, the gene-gene interactions and the microRNA analysis [24]. Therefore, we retrieved the expression data of MTAP in ovarian cancer from TCGA to verify our results. In addition, we implemented an online tool KM plotter to identify biomarkers related to ovarian cancer patients’ survival. KM plotter database is set up using gene expression data and survival information of 1648 ovarian cancer patients downloaded from Gene Expression Omnibus (GEO) and TCGA. The prognostic value was analyzed by dividing the patients into two groups according to various quantile expressions of the gene [17]. Then the PFS or OS was compared between the two groups, and...
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Figure 5. The relationship between the expression of MTAP and all the ovarian cancer patients' prognosis computed by Kaplan-Meier plotter online tool. Low expression of MTAP correlated with poor PFS and OS (MTAP, methylthio-adenosine phosphorylase; HR, hazard ratio; OS, overall survival; PFS, progression-free survival).

a Kaplan-Meier survival plot was generated with statistical significance. The relationship between MTAP and PFS and OS in ovarian cancer was analyzed in this study by this way.

In our study, the expression of MTAP was significantly down regulated in platinum-resistant ovarian cancer cell lines and tissues. Subsequently, we verified the results by computed the data extracted from TCGA database since the small sample size of our own. As was expected, MTAP was low expressed in platinum resistant patients compared to platinum sensitive patients. So we proposed that MTAP could be a potential predictor for platinum based chemotherapy response. To predict the function of MTAP and the relation with drug resistance in ovarian cancer, based on the gene/protein-gene/protein analysis and the text mining performed by the GeneMANIA and the Coremine Medical online tool, we found 54.5% of the genes interacted with MTAP were drug resistance-related genes in ovarian cancer, such as CDKN2A, CHEK2, PTEN, NFKB1 etc [25, 26]. In addition, MTAP could be involved in the regulation of drug resistance through modulating cell-growth, cell-cycle, cell-metabolism, cell-methylation etc, which have close relationship with drug resistance. Therefore, we could come up with the conclusion that MTAP was associated with the regulation of drug-resistance in ovarian cancer, especially platinum resistance.

Afterwards, we detected the relationship between the expression of MTAP and prognosis in epithelial ovarian cancer. Due to the small number of our own sample size and some patients were lost to follow-up, we did this by running on KM plotter. In all the OC patients, we found MTAP's down expression was significantly associated with ovarian cancer patients' adverse PFS and OS. When the patients were set apart according to the chemotherapy treatment, the results changed. In the platinum group, MTAP's down-regulation was correlated with poor OS and marginally with poor PFS. But when added in the paclitaxel group, that is to say, in the platinum plus paclitaxel and paclitaxel groups, its down-regulation was significantly correlated with adverse OS but no PFS. We supposed that MTAP’s down-regulation was more related to platinum treatment than paclitaxel, the data from the paclitaxel group confounded the final result. On the whole, MTAP’s down regulation had a significant relationship with an unfavorable survival prognosis, especially in the patients treated with platinum, which was consistent with previously reported results. Su etc [11] found patients with a low MTAP expression level had poor OS and disease-free survival, and the MTAP expression level retained an independent prognostic power in NSCLC. In cutaneous malignant melanoma, loss of MTAP expression was significantly associated with worse OS, and even more importantly, its expression had a clear correlation
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Over the past, many strategies were proposed to overcome MTAP-deficient tumors. There with interferon response, which could offer direct therapeutic implications [27].

Figure 6. Survival diagrams for the gene of MTAP based on Kaplan-Meier plotter online tool. Kaplan-Meier survival plots showed that low expression of MTAP resulted in a worse OS, while no apparent significance was found between low expression of MTAP and PFS (MTAP, methylthioadenosine phosphorylase; HR, hazard ratio; OS, overall survival; PFS, progression-free survival).
were experiments in vitro revealed that MTAP-deficient tumors enhanced sensitivity to inhibitors of de novo purine biosynthesis. A more recent proposal methylthioadenosine (MTA), a natural substrate of MTAP generated during polyamine biosynthesis, combined with an antimetabolite purine or pyrimidine analog, such as the guanine analog 6-thioguanine (6-TG), 5-fluorouracil (5-FU), demonstrated to be a successful application of the strategy in treating hematological tumor or solid tumor [28-30]. Therefore, MTA combined with a toxic purine or pyrimidine to treat platinum resistant patients with MTAP deficiency maybe a novel strategy in OC, which still need more studies to verify.

In the present study, we lay out various data from different points to demonstrate that MTAP has a strong connection with drug resistance of ovarian cancer and it could be a potential biomarker for predicting platinum based chemotherapy response. Furthermore, low expression of MTAP is associated with a poor prognosis in OC. However, our study also has some limitations. The sample size is relatively small; moreover, detailed mechanisms for MTAP affecting OC platinum resistance and prognosis remain unclear and require further study. Overall, MTAP may serve as an important biomarker to predict platinum based chemotherapy response and prognosis for OC patients.

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Disclosure of conflict of interest

None.

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