

REVIEW

Therapeutic targeting of methylthioadenosine phosphorylase

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Methylthioadenosine Phosphorylase is a well-known tumor suppressor and a regulator for purine and pyrimidine synthesis and metabolism. Several previous studies show MTAP could be a prognostic marker independent or coordinate with p16 in multiple cancer types. Furthermore, inhibitors of MTAP have been developed and tested in *in vitro* and *in vivo* experiment to support the selective tumor cell-killing theory of MTAP. The review aims to provide a deep understanding of the clinical role and the metabolic reprogramming regulated by MTAP in cancer as the guide to found out and solve the problems of MTAP based cancer therapy.

Keywords: Methylthioadenosine Phosphorylase; Metabolism Reprogramming; Select Killing; Target Therapy

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Introduction

In recent years, increasing evidences reveal cancer metabolite is a promising target for cancer therapeutic development. Some metabolism pathways and their intermediates crosstalk with several oncogenic signaling to control tumorigenesis and cancer metastasis. Metabolism reprogramming have been viewed as a hallmark of cancer [1]. Therefore, metabolite addiction becomes an alternative strategy particular for cancer with specific genetic defects that drive the addiction and serves as markers to identify appropriate patients for targeted therapies [2]. One-carbon metabolism is one of the metabolic pathway which has been successfully targeted in oncology. This pathway centers on folate, which acts as a single-carbon donor through initial glycine or serine for downstream nucleotide synthesis and is a major source of purine/pyrimidine synthesis [3]. Purine synthesis is one of the important source of cell growth,

energy production, and drug response. Methylthioadenosine phosphorylase (MTAP) is a gate keeper to control the balance included de novo synthesis, purine salvage synthesis, folate cycle and further regulation DNA methylation status. MTAP locates at a heritable fragile site on chromosome 9q21 and close to CDKN2A and CDKN2B which encoding both p16^{INK4a} and P14^{ARF}, specificity. MTAP usually co-deletes with CDKN in multiple types of cancer including non-small lung cancer (NSCLC) [4], osteosarcoma [5], gastrointestinal cancer [6, 7], pancreatic cancer [8] and acute T-cell leukemia [9, 10] and the decrease of enzyme activity is shown in previous research [8, 11]. MTAP plays a tumor suppressor role through regulating cell cycle [12]. Loss of MTAP function leads to tumorigenesis and is correlated with the survival in clinical cohorts [13]. In addition, hyper-methylation status at promoter also is a major modification for MTAP [14]. Previously, MTAP expression was proved can be detected through immunohisto-

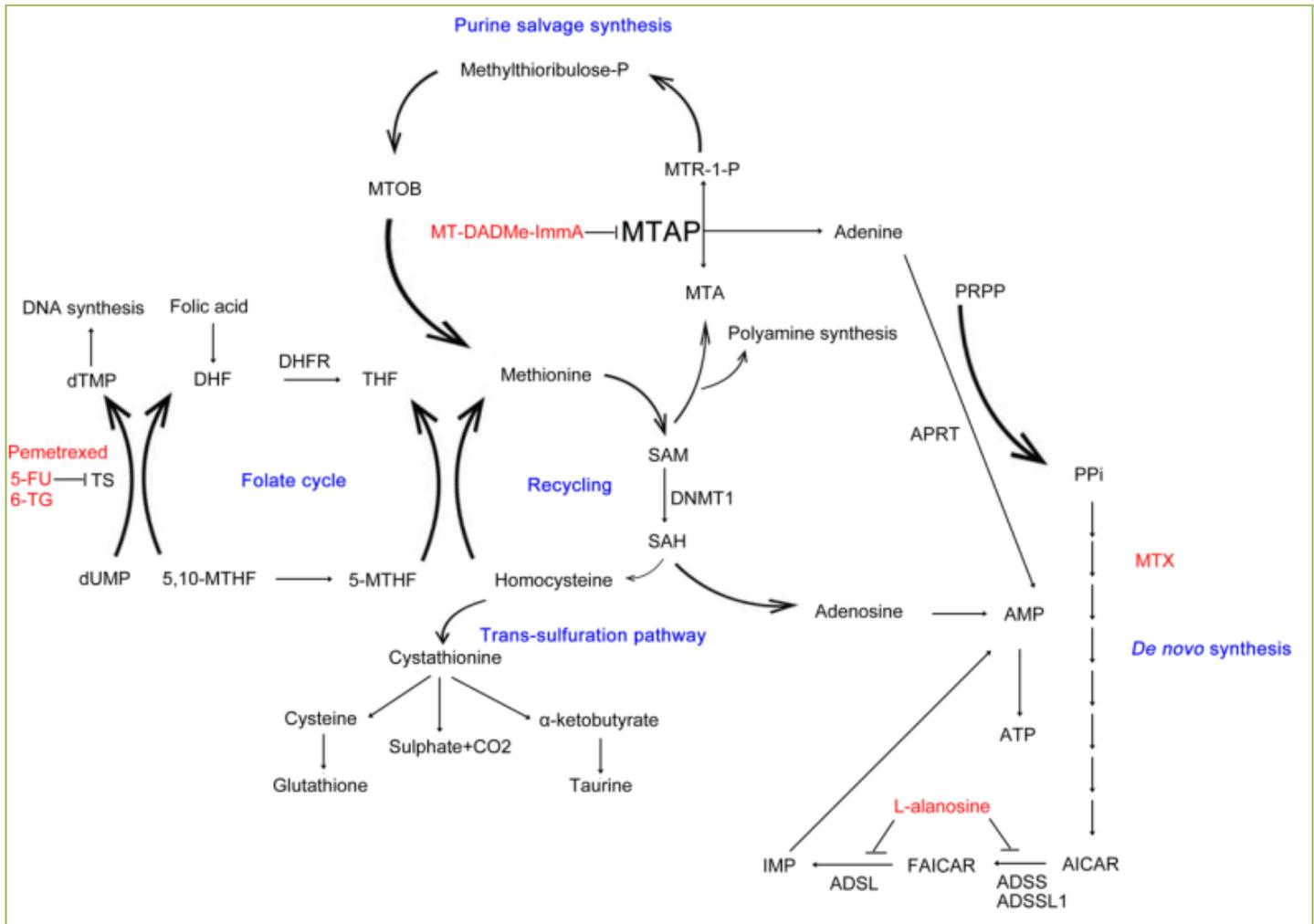


Figure 1. The diagram of adenine synthesis pathway. MTAP catalyzed the phosphorylation of MTA, be the intermediate product of polyamine synthesis. Blue color bring out the Purine synthesis pathway source from salvage pathway and *de novo* synthesis. Several metabolism reactions also interplay with adenine pathway through intermediate include folate cycle, trans-sulfuration pathway and SAM-recycling. Red colors means MTAP related inhibitor for clinical trials and corresponding targeting site.

chemistry (IHC) stain, reverse transcription-PCR and western blot to confirm its significant value in patient specimens or cancer cells. Further, next-generation sequencing (NGS) [15], fluorescence in situ hybridization (FISH) and LC-Mass were also used to detect and quantify the genetic and enzyme activity part, respectively [16]. At the beginning, MTAP just be regarded as a prognostic factor coordinated with p16, but we proofed that it is an independent prognostic maker for clinical lung cancer patients [17].

MTAP & MTA

MTAP is responsible for catalyzing the phosphorylation of 5'-deoxy-5'-methylthioadenosine (MTA) through its enzyme activity. MTA, as an intermediate during polyamine synthesis, contains the methylthioribose-1-phosphate (MTR-1P) with adenine [18]. Adenine plays the major role in generating adenosine monophosphate (AMP) rapidly. In

addition, MTR-1P then converted to downstream product methionine after series of enzymatic reactions. MTAP has high enzyme activity in wide range of normal cells, tissues and serum [19]. Controversy, MTAP activity is lost in many cancer cells and result in MTA accumulation [20]. MTA production was demonstrated to induce several matrix metalloproteinases (MMPs) secretion and activate interleukin-8 (IL-8) signaling transduction in hepatoma cells [22]. MTA accumulation also enhances activation of the NF-κB signaling [21]. Reduction of protein methylation directly interplays with SAM-recycling by MTA affects the extracellular signal-regulated kinase (ERK) activity [22]. MTA redundancy inhibits signal transducer and activator of transcription1 (STAT1) function by reduced methylation status of STAT1 [23]. MTA accumulation was also shown to enhance cell proliferation rate of stroma cell and promoted cell invasive ability by recruiting of the transcription factor activator protein-1 (AP-1) through the specific adenosine

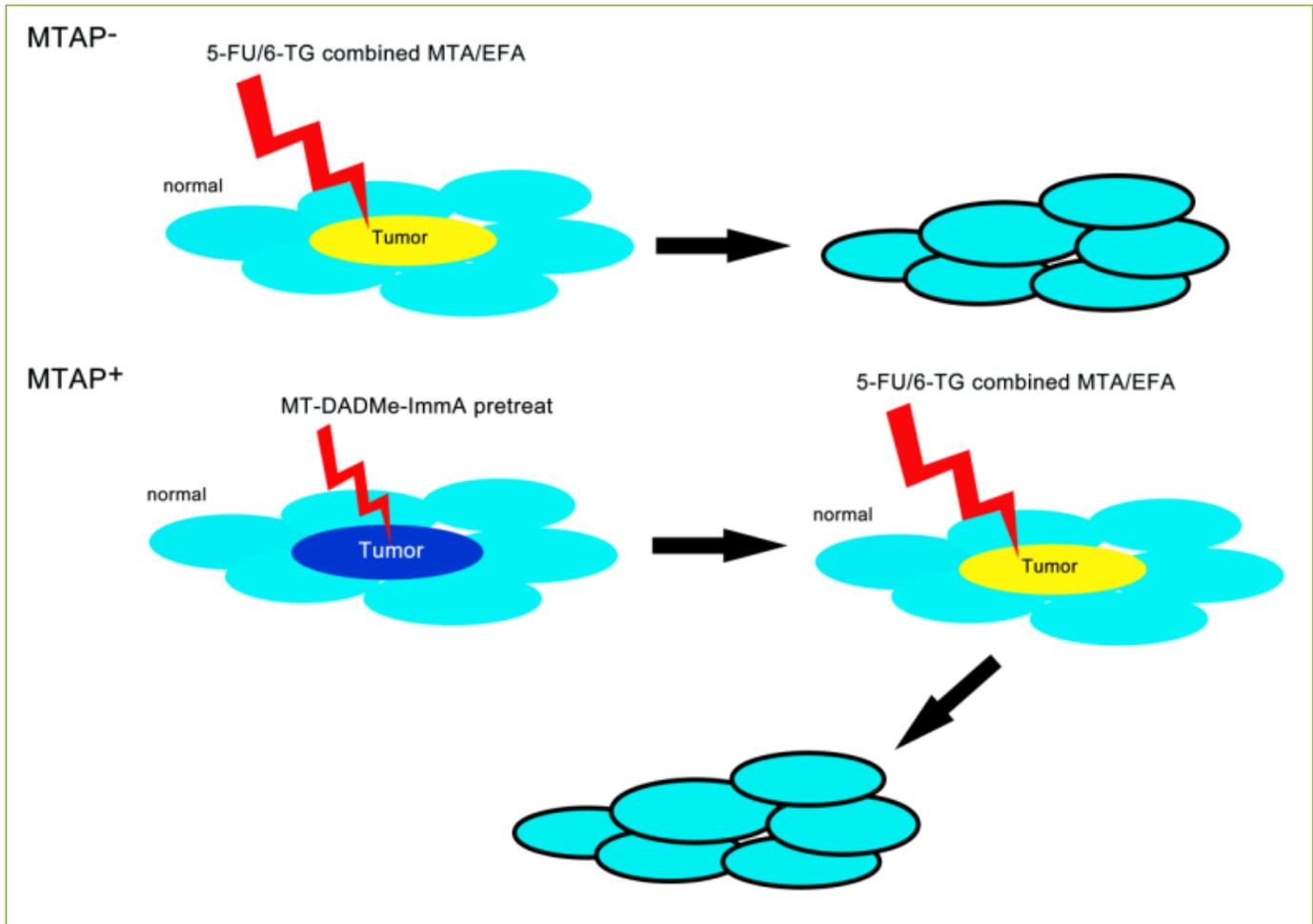


Figure 2. The proposed strategy of target therapeutics based on MTAP classification in clinical. Based on select killing theory, previously study were validate several inhibitor at difference condition. Combine all previously evidences, MTA/EFA accumulation combine chemotherapy drug 5-FU/6-TG could dramatically decreased tumorigenicity *in vivo* for MTAP negative part. And two-step treatment strategy for MTAP positive tumor. MTDIA pretreat to target population then combined with general therapy.

receptor ADORA2B transduction in melanoma [24]. However, high dose of MTA has been reported that prevented damage from liver organ in experiment model, even when MTA had been demonstrated no toxicity by long term periods by intraperitoneally injection *in vivo* [25]. Clinical trials also proposed MTA as an anti-inflammatory material could blocking melanoma and colon cancer cell growth [26]. Due to MTAP controls metabolites product including the purine, energy and methylation status. Therefore, MTAP is an important enzyme maintaining the dynamic nutrition balance in normal tissue. However, different MTAP expressions are seen in different tumors. Therefore, we review the pathways regulating the heterogeneous MTAP expression in cancer.

Salvage & De novo pathway

Purine synthesis is composed of several metabolism procedure and intermediates. From MTA through MTAP catalyzed to adenine, further adenine converted to AMP

depends on adenine phosphoribosyl transferase (APRT) activity. The process is defined as the purine salvage pathway. On the other side, methionine regard as origin source by converted MTR-IP to S-adenosylmethionine (SAM), further SAM is catalyzed to S-adenosylhomocysteine (SAH) by DNA methyltransferase 1 (DNMT1) for supplied the DNA methylation, protein synthesis and folate cycle [27]. Methionine also the essential amino acid, it's necessary to maintain normal biology and development for mammals. At the same time, SAH is hydrolyzed to homocysteine (Hcy) in a reversible reaction. Hcy could entry the trans-sulfuration pathway and also crosstalk with folate cycle by methionine synthase (MTR) and betaine-homocysteine S-methyltransferase (BHMT) catalyzed to feedback methionine looping (recycling pathway). Trans-sulfuration pathway stated at Hcy, it was been catalyzed to cystathionine by cystathionine β -synthase (CBS) and further divide of α -ketobutyrate, sulfate and cysteine. In addition, de novo purine synthesis consist of the

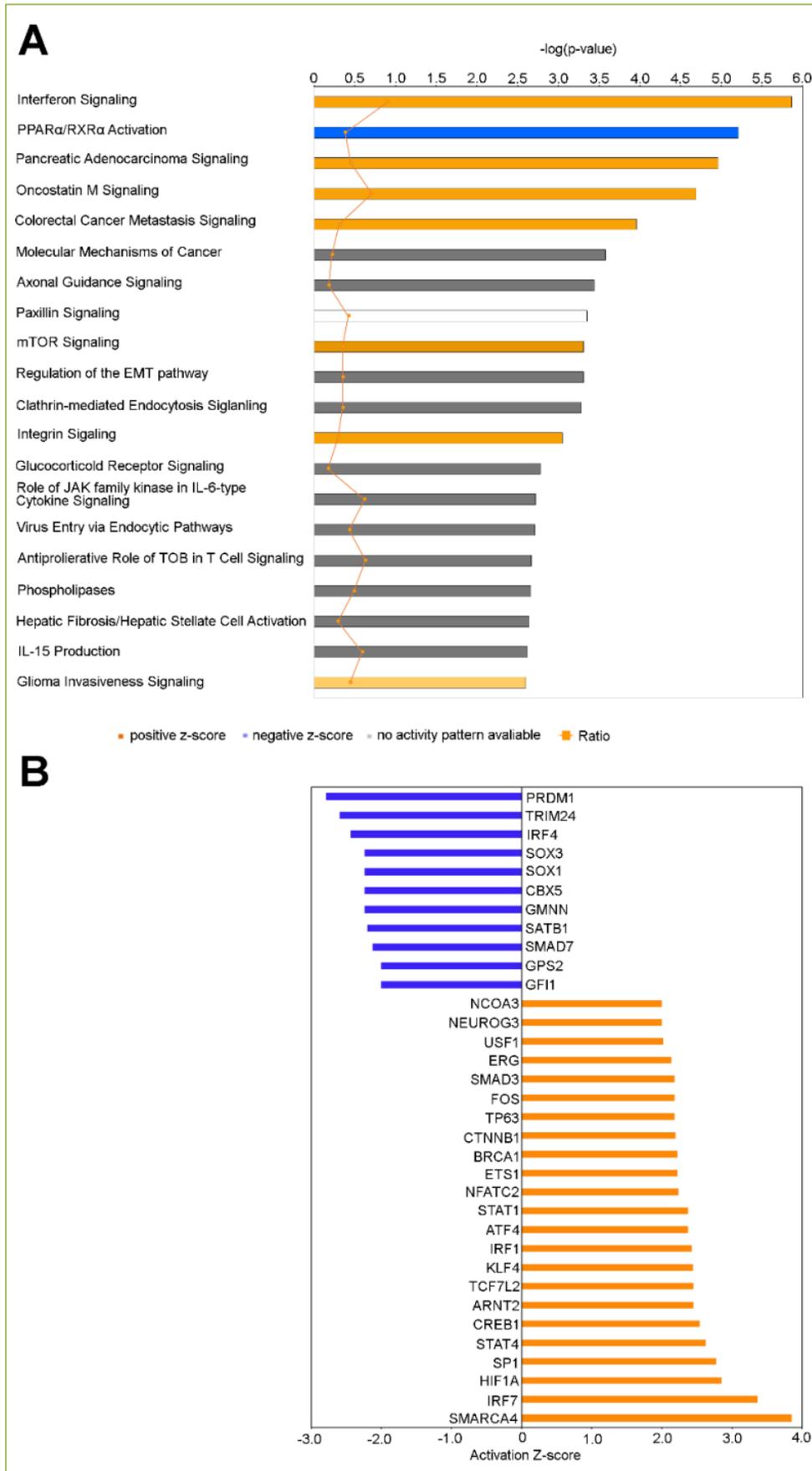


Figure 3. *In silico* analysis the transcriptomics data of MTAP expression. (A) Prediction of the canonical pathway that involving the MTAP positive versus negative through IPA analyzed. Interferon signaling as regard as most significant activation pathway from MTAP-based transcriptomics data. On the other hand, PPARα were be inhibited when MTAP express. (B) Prediction of transcription factors that involving the MTAP positive versus negative through IPA analyzed. The orange part means that factors will be activation when MTAP overexpress, and several transcription factors will be inhibited (blue part) at the same condition.

precursor product (PRPP) and 11 step-related enzymes and 10 intermediate to final result AMP production. PRPP interacts with APRT and catalyzes the (PRA) and PPI. The enzymes including 5-aminoimidazole-4-carboxamide ribonucleotide formyl transferase (ATIC), adenylosuccinatelyase (ADSL) and adenylosuccinate synthase (ADSS) which were reported to be correlated with tumorigenesis. The above evidences point out that MTAP is a unique and specific factor both in normal and cancer side.

Inhibitors

Due to purine pathway and MTAP activity have their values and potentials for clinical target therapy. So, several drugs and analogs have been developed to use according to MTAP status of tumor^[28]. Base on previous study and providence, scientists design some inhibitors to block single typing of MTAP. L-alanosine, MTX and methylthioDADMe-Immucillin-A (MTDIA) are commonly used for related experiment and they defined difference means for MTAP-induced cancer^[29].

L-alanosine

Pre-clinical trials combine with L-alanosine are used in MTAP-deficiency cancer^[30]. Previous data showed that MTAP-deleted tumors lack the ability to salvage adenine and thus rely on de novo purine biosynthesis for AMP production. As a result, MTAP tumors are particularly sensitive to L-alanosine, an inhibitor targets to the final two steps involving conversion AMP from inosine monophosphate (IMP) corresponding enzymes ADSS and ADSL, specificity. Unfortunately, L-alanosine has unremarkable effectivity for patients with MTAP-deficient tumors by a phase II study^[31].

MTX

Methotrexate (MTX) has been reported to increase sensitivity in MTAP negative type. MTX could inhibition of purine de novo synthesis through its anti-purine actions and bypass to anti-folates^[32]. It has similar function with L-alanosine, but different targeting. MTX could decrease the source uptake for de novo synthesis instead of decreasing enzyme activity of ADSS and ADSL^[33]. Previous data also indicate that IFN overexpression could increase the MTX sensitivity in MTAP-deficient tumor^[33].

MTDIA

MethylthioDADMe-Immucillin-A (MTDIA) has been developed as a transition state analogue for MTAP inhibitor^[34]. Previous data showed MTDIA could induce cell apoptosis, inhibit cell proliferate rate *in vitro* and

tumorigenesis ability *in vivo* in lung cancer model included A549 and H358 cell lines that MTAP express negative and positive, respectively^[35]. Based on the theory and model, the results showed MTDIA could focus on MTAP expressing cancer, hence, combining MTDIA with L-alanosine or MTX could be a two-step therapeutic strategy.

In recent year, single use of inhibitors did not significant prolong patients survival rate and strong corresponding side effect by enzymatic-base blocked. The new strategy combining enzymatic-base therapy and chemotherapy drugs which was developed as purine and pyrimidine analogs such as (5-FU) and 6-thioguanine (6-TG). 5-FU is an anti-pyrimidine and is widely used in the clinical cancer patients^[25]. On the other hand, 6-TG inhibits *de novo* purine synthesis^[36]. However, the previous study showed the specific inhibitor not only regulates its expression level, but also decreases the enzymatic activity to induced unexpectedly side-effect at the clinical part.

Conclusions

Through *in silico* analysis, we try to find out the novel signature from microarray analysis of GSE56112, which includes MTAP positive/negative gene expression in fibrosarcoma cell and reverses the MTAP expression by MTAP inhibitor MTDIA. Initially, we set 1.5 fold change cut-off by clustered 3-independent duplicate of MTAP⁺ compared with MTAP⁻ through GeneSpring software. We further analyzed the candidate genes involving canonical pathway and upstream factors by IPA (Ingenuity Pathway Analysis) prediction. The results are consistent with a previously study which showed IL-8, interferon and NF- κ B related signaling pathway were upregulated when MTAP overexpress^[37]. In the meantime, several transcription factors were also activated including STAT1 and AP-1. In addition, the prediction also showed some novel signatures and factors in MTAP-based transcriptomics. KLF4 and SOX family members reflected MTAP may play a regulator role of cell stemness. HIF-1 α , ARNT and MYC also revealed MTAP overexpression could induce metabolic reprogramming in tumorigenesis. Interestingly, some pathways were not reversed by MTDIA treatment in IPA analysis. This result suggests that MTAP still has an unknown enzymatic independent function involving tumorigenesis. In addition, whether inhibitors regulating MTAP enzyme activity could induce imbalance of purine/pyrimidine synthesis is still unknown. Previous study also mentioned the importance of non-enzymatic function of metabolism-related genes^[38, 39]. Therefore, we need to establish MTAP-based proteomics and phosphoproteomics database to find out the key component interacted with MTAP. Recently, the crystal structure of MTA-bound

PRMT5 indicated that MTA alters the cofactor binding pocket and competed with SAM for binding to PRMT5^[40, 41]. MAT2A, the PRMT5 substrate, was downregulated in MTAP deficient cells^[42]. Furthermore, through designing or virtual screening the specific compound or drug for validating the dock control interaction status and further evaluating whether it has the value of therapeutic potential.

Conflicting interests

The authors have declared that no conflict of interests exist.

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