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Review Article

The Anticancer Effects of S-Adenosylmethionine on Breast Cancer Cells

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Submitted: 15 November 2017

Accepted: 06 December 2017

Published: 08 December 2017

ISSN: 2333-6633

Keywords
- S-Adenosylmethionine
- Human breast cancer cells
- Growth inhibition
- Apoptosis
- Autophagy

Abstract

S-Adenosyl-L-methionine (AdoMet) is a naturally-occurring sulfonium compound found in almost every tissue and fluid in the body that plays a central role in cellular metabolism. AdoMet, indeed, is the link to three key metabolic pathways: polyamine synthesis, transmethylation and transsulfuration. Literature and clinical studies regarding the AdoMet physiologic and physio-pathological roles underline its therapeutic potential to treat alcoholic liver disease, depression and joint pain. In recent years, several new metabolic functions have been assigned to this important and widely occurring sulfonium compound that exerts pleiotropic effects on signal transduction in many and different cell-types. In the last decade a lot of *in vitro* and *in vivo* studies highlighted the anti-proliferative, anti-metastatic and pro-apoptotic effects of AdoMet in cancer cells. In this review we summarize the most recent studies focusing on the anticancer effect of AdoMet in breast cancer cells and provide information on the broad spectrum of mechanistic actions underlying the antitumor effect of this physiological sulfonium compound. Our data on the synergistic effects of AdoMet with conventional therapeutic drugs appear to be clinically relevant and disclose a new scenario of intervention in which this natural and safe molecule, alone or in combination with other chemotherapy drugs could be utilized for the development of novel promising therapeutic strategies for the treatment of breast cancer.

ABBREVIATIONS

AdoMet: S-Adenosyl-L-Methionine; 5-azaCdR: 5-Aza-2'-Deoxycytidine; *BRCA*: Breast Related Cancer Antigens; cFLIP: FLICE-Like Inhibitory Protein; CLC: Chloroquine; Doxo: Doxorubicin; IL: Interleukin; MMP2: Matrix Metalloproteinase-2; MTA: 5'-deoxy-5'-Methylthioadenosine; MT1-MMP: Membrane Type 1 Matrix Metalloproteinase; PARP: poly (ADP ribose) Polymerase; TIMP-2: Tissue Inhibitor of Metalloproteinase-2; uPA: Urokinase-Type Plasminogen Activator; VIDAZA: 5-Azacytidine

INTRODUCTION

S-Adenosyl-L-methionine (AdoMet, also abbreviated with the acronym SAM or SAMe) is a widely occurring sulfonium compound that plays a primary role in cellular metabolism since it is involved in a wide variety of important biochemical processes [1-6].

The peculiar AdoMet biochemical as well as chemical properties are inherent in its sulfonium pole susceptible to nucleophilic attack, and therefore leading to molecular instability [1,2]. The unique AdoMet reactivity, in fact, is due to the presence of a sulfonium ion that makes the three carbon atoms (bound to the sulfur atom) highly susceptible to nucleophilic substitution; this renders the molecule extremely reactive and able to donate the methyl group, aminopropyl group and adenosyl group (Figure 1). AdoMet and S-adenosyl-(5')-3-methylthiopropylamine, its decarboxylated product, represent the only sulfonium compounds detectable in mammalian tissues [1,2].

The central role of AdoMet in cellular metabolism is well known since its discovery by Giulio Cantoni, in 1952 [7]. The sulfonium compound, in fact, represents the most versatile donor of methyl groups and is also the precursor of decarboxylated AdoMet, the donor of the propylamine group in polyamine biosynthesis. In addition, AdoMet is involved as donor of the lateral chain in the biosynthesis of diphthamide, ethylene and in several post-translational modification reactions during tRNA biosynthesis. Furthermore, AdoMet is able to donate the NH_2-group of the lateral chain during the biotin synthesis as well as the whole adenosyl portion. The sulfonium compound can also function as allosteric modulator in several enzymatic reactions [1,2]. In addition to multiple functions performed by AdoMet in cellular metabolism, recently many *in vitro* and *in vivo* studies have shown the involvement of the sulfonium compound in various cellular processes, including proliferation, differentiation, cell cycle regulation, and apoptosis [8-13]. Most

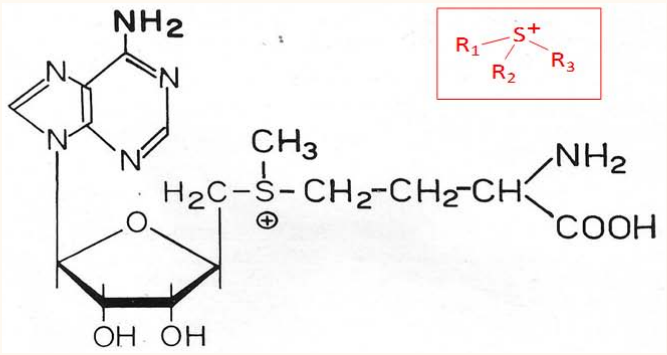

Figure 1 Chemical structure of S-adenosylmethionine and sulfonium ion (red).

of the scientific papers in the literature report data on liver, hepatocarcinogenesis and hepatocellular carcinoma.

Apart from AdoMet action on liver cancer cells, recent studies demonstrated that AdoMet is able to halt the progression of many other human tumors, albeit with different mechanisms.

Zhao Y et al., have shown that AdoMet exerts an inhibitory effect on the growth of human gastric carcinoma both *in vivo* and *in vitro*. It was reported that AdoMet treatment inhibited, in a dose- and time-dependent manner, the growth of SGC-7901 and MKN-45 gastric cells by reducing the expression of urokinase-type plasminogen activator (uPA) and *c-myc* genes. *In vivo* AdoMet treatment reduced the volume of gastric xenografts tumors in male Balb/c nu/nu mice. It was also demonstrated that AdoMet down-regulated the expression of uPA and *c-myc* genes by partly or completely methylating these genes [14].

It has been shown that in MGC-803 human gastric cancer cells and in HT-29 colon carcinoma cells, *c-myc* and *H-ras* oncogenes are hypomethylated and their expression level is particularly high. AdoMet treatment induces methylation in the promoter of such oncogenes, inhibiting protein expression with consequent reduced tumorigenesis. It is interesting to note that non-cancerous cells do not present changes in the expression of c-myc and H-ras after AdoMet treatment [15].

Li et al., report that AdoMet treatment of RKO and HT-29 colon carcinoma cells reduces the expression of several anti-apoptotic genes, such as the FLICE-like inhibitory protein (cFLIP) coding gene [16]. cFLIP is a protein homologous to caspase 8 but lacks of a catalytic domain. Therefore, cFLIP does not show protease activity but can dimerize with caspase 8, forming an inhibitory complex that limits apoptosis. AdoMet treatment also promotes the activation of caspase 8 which in consequence activates Bid protein through a proteolytic cut, responsible for the cytochrome c release from the mitochondria and for apoptosome formation [16].

Recently, the involvement of AdoMet in regulation of genes responsible of cell invasion and metastasis has also been elucidated [17]. In highly invasive SW-620 colorectal cancer cell line, AdoMet exerts anti-proliferative and anti-invasive effects through the hypermethylation of genes involved in the metastatic process. Hussain et al., showed that the treatment with the sulfonium compound causes the inhibition of the matrix metalloproteinase-2 (MMP2) and of membrane type 1 matrix metalloproteinase (MT1-MMP) mRNA levels together with an up-regulation of the tissue inhibitor of metalloproteinase-2 (TIMP-2) [17]. MMPs belong to a family of structurally related proteolitic enzymes that facilitate degradation of extracellular matrix and the basement membrane.

It has been also reported that AdoMet and its metabolite 5'-deoxy-5'-methyltioadenosine (MTA) reduce the inflammation-induced colon cancer by inhibiting fundamental pathways involved in colon carcinogenesis [18]. In Balb/c mice with drug-induced colon cancer, AdoMet and MTA treatment reduced tumor load of about 40%. In this *in vivo* study both molecules induced apoptosis and inhibited cell proliferation by targeting β-catenin, NFkB, and interleukin-6 (IL-6) signaling and by reducing STAT3 and AKT phosphorylation. The inhibition of IL-6 signaling was also studied in Colo205 colon cancer cells were it has been demonstrated that AdoMet and MTA are able to reduce the expression and transcription of IL-10, the target gene of IL-6 [18].

In PC-3 prostate cancer cells, AdoMet treatment is able to suppress the expression of genes such as uPA and MMP-2, involved in tumor progression, invasiveness and metastasis formation, by increasing their methylation [19]. Moreover, inoculation of AdoMet-treated PC-3 cells in male Balb/c nu/nu mice resulted in the development of tumors with a smaller volume than that of animals inoculated with PC-3 cells transfected with vehicle alone [19].

It has been recently demonstrated that in human LM-7 and MG-63 osteosarcoma cells AdoMet treatment leads to a dose-dependent decrease of cell proliferation and invasiveness of tumor cells by inhibiting the expression of the genes involved in the formation of metastasis, angiogenesis and cellular invasion [20].

In addition, we have reported that the sulfonium compound strongly inhibits the proliferation of U2OS osteosarcoma cells by slowing-down cell cycle progression and by inducing apoptosis. In this model system we found that AdoMet consistently causes a reduction in protein expression levels of cyclin D and E, an increase of p53 and p21 cell-cycle inhibitor and a marked increase of pro-apoptotic Bax/Bcl-2 ratio [21]. Furthermore, the AdoMet-induced antiproliferative effects were dynamically accompanied by profound changes in protein and phosphorylation levels of ERK1/2 and STAT3 [21].

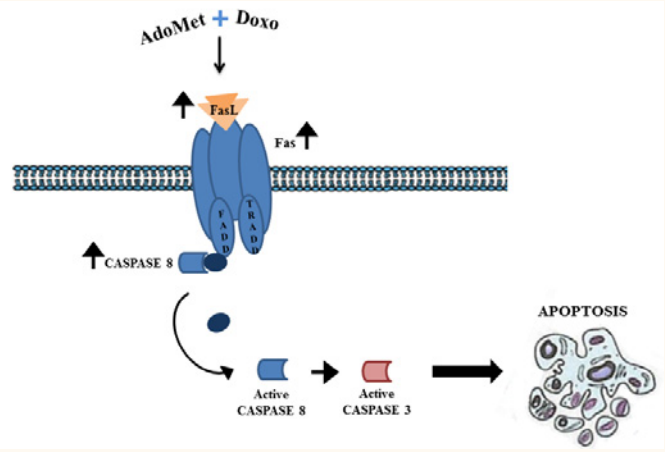

Figure 2 Proposed death receptor-associated apoptosis as mechanism underlying the synergistic antitumor effect of Doxo and AdoMet in CG5 breast cancer cells.

The effect of exogenous AdoMet on the osteosarcoma progression has been investigated in the osteosarcoma mouse model [22]. It has been demonstrated that in AdoMet-treated mouse there is a reduced cell proliferation especially in primary osteosarcoma but not in metastatic form of osteosarcoma. Moreover, the antiproliferative effect exerted by the sulfonium compound is associated with a lower expression of Sox2 and with an up-regulation of translationally controlled tumor protein, both proteins linked to the proliferative ability of normal bone tissue, suggesting that AdoMet is effective in the primary stage of osteosarcoma [22].

It has been very recently demonstrated that AdoMet is able to enhance the antitumor effects of selenomethionine, methylselenocysteine and methylseleninic acids in human cervical cancer HeLa cells where AdoMet, in combination with the selenium compounds, synergistically inhibits cell proliferation, migration and adhesion by affecting ERK and AKT signaling pathways [23].

Despite in recent years growing evidence has accumulated in the literature on the anti-proliferative, anti-metastatic, and pro-apoptotic effects of AdoMet in cancer cells [8-23], a clear understanding of the complex biological mechanisms exerted by AdoMet as well as its possible clinical applications are far to be defined.

In this article a comprehensive overview of the significant antitumor effects exerted by AdoMet in breast cancer cells is reported and the possible underlying mechanisms are discussed.

Breast cancer is the leading cause of death in women [24-27]. About 1.3 million cases of invasive breast cancer are diagnosed, and it affects mainly women over 45 years old. Breast tumor occurs also in men, but it is more than 100 times less common. Therapies currently used in the treatment of breast cancer are very personalized and depend on several factors, including the tumor's subtype, the stage of the tumor, genomic markers, the patient's age, general health, menopausal status, and also the presence of known mutations in inherited breast cancer genes, such as *BRCA1* or *BRCA2* [28,29]. The metabolic facet of *BRCA1/2* might involve tissue-specific alterations in AdoMet, critical factor for methylation dynamics in the nuclear epigenome. This in turn might induce faulty epigenetic reprogramming directing cell-specific differentiation of breast cells, which can ultimately determine the penetrance of *BRCA* defects [30]. Despite the recognition of these risk factors, approximately 70% of women who develop breast cancer do not have any identifiable risk factor.

S-ADENOSYLMETHIONINE AFFECTS TUMOR PROGRESSION, INVASIVENESS AND METASTASIS FORMATION

Breast cancers usually are epithelial tumors of ductal or lobular origin, which are classified into *in situ* carcinoma and invasive (infiltrating) carcinoma, characterized by specific growth patterns and cytological features [24-27]. The dissemination of cancer cells to distant sites is linked with poor patient prognosis and metastatic diseases, which represent a vast percentage of cancer patient mortality [31]. The metastatic process consists of a series of sequential, interrelated steps including tumor cells detachment from the primary tumor, increased motility and invasion, proteolysis, and resistance to apoptosis.

The invasion process starts when tumor cells acquire the ability to penetrate the surrounding tissues, and these motile cells pass through the basement membrane and extracellular matrix, penetrating into the blood and lymphatic vessels [31-32].

DNA methylation, an epigenetic modification, represents one of the most studied epigenetic processes in breast cancer [33,34]. DNA methyltransferases are the key enzymes for DNA methylation and catalyze the transfer of a methyl group from AdoMet to cytosine, thus forming 5-methylcytosine. Methylation of CpG islands in the promoter region of a gene might inhibit the access of the transcriptional machinery to chromatin, thus silencing gene expression. The carcinogenesis is normally associated with hypomethylated pattern of the cytosine methylation in CpG dinucleotides in regulatory regions of genes important for cancer transformation, development and invasion [35-37].

Data accumulated over the last two decades have established that the methylation of genes responsible of cell invasion and metastasis is a potential therapeutic target in cancer [35-37].

In this contest, a Canadian group has studied in depth the epigenetic regulation induced by AdoMet on uPA and MMPs proteins in breast cancer cells [38,39].

uPA is an extracellular serine protease involved in the process of tumor invasion and metastasis. uPA catalyzes the conversion of the extracellular zymogen plasminogen to the active matrix-degrading protease plasmin, thus increasing cancer cell invasion into surrounding tissues, directly by degrading the basement membrane and extracellular matrix proteins or indirectly, by activating other pro-enzymes such as pro-MMP [40].

MMPs are a family of secreted, zinc-dependent endopeptidases and are involved in tissue-remodelling processes, including wound healing, embryo implantation, tumor invasion, metastasis, and angiogenesis [41].

In a work published in 2004, Pakneshan and colleagues analyzed the effects of AdoMet treatment on three different lines of highly invasive breast cancer cells: MDA-MD-231, BT549 and HS578T [38]. These cells are characterized by high level of uPA expression and by demethylation of uPA promoter.

They demonstrated that AdoMet treatment for 6 days strongly inhibited uPA mRNA expression and its enzymatic activity. This inhibition was due to the hypermethylation of CpG sequences in the uPA promoter, thus causing gene silencing and inhibition of invasive capacity of these cells.

In a work published in 2013, Chik and colleagues studied the anticancer effects of AdoMet and 5-azacytidine (VIDAZA) or its deoxy-analogue (5-azaCdR) combination on non-invasive MCF-7 and ZR-75-1 and on invasive MDA-MB-231 breast cancer cell lines [39]. The VIDAZA is an epigenetic drug that inhibits DNA methylation, thus causing cancer growth inhibition while it could also induce cancer invasiveness through the inhibition of uPA and MMP2 promoter methylation [39]. They tested whether the combination of the 5-azaCdR with AdoMet would block the adverse demethylating activity of 5-azaCdR while maintaining its growth suppression effects. In non-invasive human breast cancer cells MCF7 and ZR-75-1, it has been demonstrated that AdoMet inhibited global and gene specific demethylation induced by 5-aza-CdR and prevented 5-aza-CdR activation of pro-metastatic genes uPA and MMP2. This brought to inhibition of cell invasiveness while increased the growth inhibitory effects of 5-azaCdR and its effects on tumor suppressor genes as p21. In contrast to the result obtained in MCF-7 and ZR-75, in highly invasive breast cancer line MDA-MB-231 the combination of 5-aza-CdR with AdoMet was synergic in blocking the cell invasiveness through a significant suppression of uPA expression.

In summary, pharmacological administration of the methyl donor AdoMet, which is a natural and safe molecule, due to its ability to methylate and therefore to silence pro-metastatic genes, could be used as a promising therapeutic approach for blocking breast cancer cells progression into metastasis.

S-ADENOSYLMETHIONINE AFFECTS CELLS PROLIFERATION AND POTENTIATE DOXORUBICIN IN INDUCING APOPTOSIS

Our research group has deeply investigated AdoMet antiproliferative effect on different hormone-dependent and -independent breast cancer cell lines and has proposed the possible cell death mechanism responsible for the synergistic effect of the combination of the sulfonium compound with doxorubicin (Doxo), one of the most used anticancer drug [42].

Drug combinations in cancer therapy could be useful to improve clinical responses and emphasize the anti-tumor activity of single molecules.

Doxo and other anthracycline are canonical drugs for breast cancer and therefore currently used in antitumoral treatments. Unfortunately, resistance to these agents is common, representing a major obstacle to successful treatment. Moreover, the benefits in response rate and overall survival, however, are often associated with myelosuppression and cardiomyopathies [43]. In this context, the combination of Doxo with natural molecules with antiproliferative properties, such as AdoMet, could be useful in order to lower drug concentration, thus reducing the side effects and increasing anthracycline efficacy.

Specifically, we have evaluated the growth inhibition induced by different concentrations of AdoMet in combination with

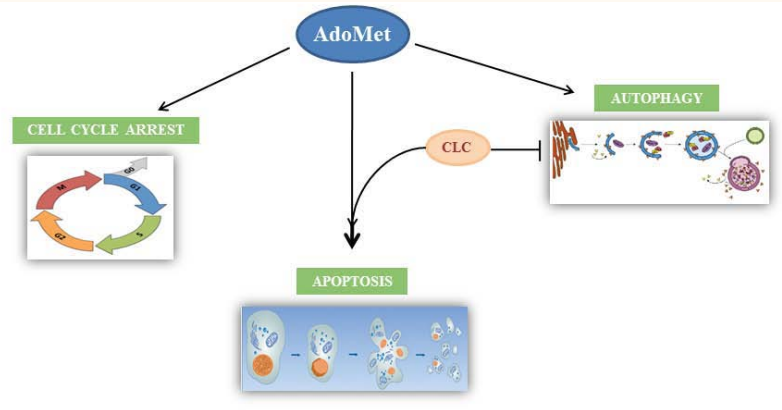

Figure 3 Antitumor effect of S-adenosylmethionine on MCF7 cells. AdoMet inhibits cell proliferation, induces autophagy and synergizes with chloroquine in inducing apoptosis.

Doxo after 72 hours treatment of CG5, MCF-7, and MDA-MB 231 cells [42]. We have found that the combined effect of the two molecules at equitoxic concentrations was highly synergistic in CG5 and MCF-7 cells, while it was only additive or lightly synergistic in MDA-MB 231 cells. The mechanisms of cell death induced by the synergistic combination AdoMet/Doxo has been evaluated in hormone-dependent CG5 cells, with special interest upon programmed cell death. It was shown that AdoMet is able to potentiate the apoptosis induced by Doxo alone, thus suggesting that the synergism on growth inhibition was largely due to apoptosis. Notably, the AdoMet/Doxo combination induced a significant activation of caspases 3, and 8, while no effect was found on caspase 9 cleavage, thus indicating the triggering of the death receptor-associated apoptotic pathway, mediated by caspase 8 as initial caspase and caspase 3 as terminal caspase. In contrast, in cells treated with AdoMet and Doxo alone no significant variations of the expression of cleaved caspase 8 and of the mitochondria-associated caspase 9 were observed.

These experimental evidences suggest that the molecular mechanism underlying the synergistic effect of AdoMet and Doxo in the regulation of hormone-dependent breast cancer cell proliferation consisted in the induction of the extrinsic apoptotic pathway rather than the intrinsic mitochondrial pathway, or other forms of cell death such as autophagy or oxidative stress, in which instead Doxo seems to give a greater contribution.

Fas/FasL system is a key signal pathway involved in apoptosis induction in several different cell lines [44]. Two types of Fas-mediated signaling pathways have been reported, in both of which caspase-8 activation represents the downstream event [45,46]. It has recently been proposed that Doxo-induced apoptosis is mediated by the activation of the Fas/FasL system [47]. Moreover in recent reports it has been proposed that Fas-mediated apoptotic pathways could be regulated by the activity of the enzyme that synthesizes AdoMet (MAT II) and by AdoMet-dependent transmethylation mechanism [48]. Recently it was also reported that FasL and caspase-8 expression could be regulated by epigenetic mechanisms [39].

In Figure (2) is schematically summarized the proposed mechanism responsible for the synergistic effect of AdoMet/Doxo combination in cell death of hormone-dependent CG5 breast cancer cells. AdoMet, in combination with Doxo greatly potentiates the pro-apoptotic effect of the drug resulting in the increased expression of the cell death ligand FasL and cell death receptor Fas followed by the recruitment of procaspase 8. This process gives rise to caspase 8 activation which in turn induces apoptosis by directly activating caspase 3.

This study highlight the importance of the synergistic effect of AdoMet with Doxo in the regulation of hormone-dependent breast cancer cells proliferation and emphasizes the antitumor activity of the sulfonium compound.

INHIBITION OF AUTOPHAGY BY CHLOROQUINE ENHANCES THE ANTITUMOR EFFECT OF S-ADENOSYLMETHIONINE BY PROMOTING APOPTOSIS

Despite our knowledge on the roles played by AdoMet on tumor cell growth, the molecular mechanisms underlying the anticancer properties of the sulfonium compound are not still well defined. To better elucidate the complex biological effects exerted by AdoMet, in-depth studies have been recently conducted by our research group utilizing hormone-dependent MCF-7 breast cancer cells [49]. In this model system we have demonstrated that AdoMet treatment causes cell growth inhibition and that this effect was due to the alteration of cell cycle and to the induction of apoptosis. In particular, AdoMet induced a cells blockage at G2/M phase, a reduction in the expression levels of cyclin B, D and E, and an increase in the expression levels of p53 and cell cycle-inhibitors p21 and p27.

We also demonstrated that AdoMet induces apoptotic cell death in a dose- and time-dependent manner, and that this effect is accompanied by a significant increase of pro-apoptotic Bax/Bcl-2 ratio, paralleled by poly (ADP ribose) polymerase (PARP) and caspase 6 and 9 cleavage. It is worth noting, in this respect, that the increased expression of the antiapoptotic protein Bcl-2 was correlated to chemo-resistance, and that its targeting is an emerging strategy in cancer treatment [50].

An important mechanism of escape from apoptosis is the induction of autophagy, a self-degradation process that plays an important role either in maintaining cell function or in inducing cell death [51-53]. Despite in recent years many studies have investigated on the role of autophagy in various diseases, the molecular mechanisms underlying the regulation of this process in cancer cells are not completely understood [51-53]. Autophagy seems to be clearly associated with chemotherapy resistance. Therefore, the pharmacological modulation of this process appears to have significant clinical potential as a novel therapeutic anticancer strategy. On this basis, several preclinical and clinical studies aim to inhibit the autophagic process by means of different inhibitors. Chloroquine (CLC), an antimalarial drug largely used in clinical settings, has been recently recognized for its ability to block autophagy by inhibiting autophagosome-lysosomal fusion event [53,54].

We demonstrated for the first time, at least at the best of our knowledge, that AdoMet is able to induce the autophagy process, and that this event is associated with a significant increase, both at qualitative and quantitative level, of beclin 1, LC3B cleavage and autophagosome formation, which represent important markers of autophagy activation [53,55-57].

We hypothesized that, in MCF-7 cells, autophagy could acts as an escape mechanism from the pro-apoptotic activity of AdoMet. To verify this hypothesis we assessed whether inhibition of autophagy through the use of CLC could modulate the antiproliferative effect of the sulfonium compound on breast cancer. Consistently with this hypothesis, the AdoMet/CLC combination greatly enhanced apoptosis induced by AdoMet, as highlighted by the increased cleavage of caspase 6 and PARP. Furthermore, the co-treatment appeared to be synergistic in the inhibition of phosphorylation of AKT/mTOR kinases, two key regulators of cell metabolism often dysregulated in cancer [58,59]. In breast cancer, mutations in EGFR/PI3K/PTEN/AKT/mTORC1 pathway play a fundamental role in tumor genesis and tumor progression, resulting in AKT and mTOR activation. In clinical settings, AKT inhibitors have been used in combination

with CLC, and they showed synergistic effect in inducing cell death in cancer [60-62].

In this scenario, the enhancement of the antiproliferative effect of AdoMet through a combined treatment with autophagy inhibitors, such as CLC, could represent an effective new strategy to be used in the future also in clinical setting to shut down AKT/mTOR pathway.

In conclusion, the effects of AdoMet on MCF-7 cells, schematically summarized in Figure (3), suggest that in breast cancer cells this physiological compound can serve as a viable and attractive anticancer agent triggering mechanisms that lead to cell growth arrest and apoptosis activation. Moreover, the experimental evidence that autophagy can act as a survival mechanism from the apoptotic activity of AdoMet provides the basis for a possible use of the sulfonium compound in combination with autophagy inhibitors in order to improve the pharmacological therapy of breast cancer.

CONCLUSIONS

The potential of AdoMet as antiproliferative agent has been evidenced in the literature, and growing scientific interest is focused on identifying the biological mechanisms and the signal transduction pathways related to the chemo-preventive activity of this physiological compound.

The high incidence of breast cancer in developing countries and its poor prognosis partially attributed to multiple-drug resistance and anti-apoptosis activity of cancer cells has prompted scientists to discover more effective and less toxic therapeutic and preventive strategies able to interfere with the recurrent phenomenon of resistance to hormonal and targeted therapy that represents the first-line treatment in the management of breast cancer patients.

The present review summarizes the most recent studies focusing on the anticancer effect of AdoMet in breast cancer cells and provides information on the broad spectrum of mechanistic actions underlying the antitumor effect of this physiological sulfonium compound already available as an approved nutritional supplement.

S-Adenosylmethionine, due to its ability to methylate and therefore to silence pro-metastatic genes, is able to affect tumor progression, invasiveness and metastasis formation. AdoMet synergistically potentiates the antitumor effect of Doxo in the regulation of hormone-dependent breast cancer cell proliferation through the activation of the death receptor-associated apoptosis. Finally, AdoMet in combination with CLC modulates the process of autophagy that represents an important mechanism of escape from apoptosis thus providing the possibility to improve the pharmacological therapy of breast cancer. At the same time, our findings open new questions on the mechanism of synergism between CLC and AdoMet, which seems to be related not only to autophagy inhibition, as it happens in other published researches, but also to the inhibition of AKT/mTOR signaling.

Collectively, these results provide evidences that in breast cancer cells AdoMet acts as a potent anticancer agent able to block various tumor-promoting genes and signaling pathways and can therefore be considered as a promising and attractive target for further investigations finalized to the design of innovative adjuvant therapies in breast cancer treatments.

It is interesting to note that AdoMet is available as a dietary supplement in the United States since 1999 and pharmaceutical preparations of this compound are available as intravenous, intramuscular, and oral forms. Reviews of clinical studies to date indicate that, at pharmacological doses, AdoMet has a low incidence of side effects with an excellent record of tolerability. Moreover, no toxic or antiproliferative effects have been reported in normal, non tumorigenic cells [63]. Thus, it is conceivable that the concentrations of AdoMet that would inhibit cancer cell proliferation, utilized in our as well as in other studies, could be useful for further trials in patients.

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Cite this article

Cave DD, Ilisso CP, Mosca L, Pagano M, Martino E, et al. (2017) The Anticancer Effects of S-Adenosylmethionine on Breast Cancer Cells. JSM Chem 5(3): 1049.