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Methylseleninic acid promotes antitumour effects via nuclear FOXO3a translocation through Akt inhibition

Míriam Tarrado-Castellarnau[a,b], Roldán Cortés[a,b], Miriam Zanuy[a,b,1], Josep Tarragó-Celada[a,b], Ibrahim H. Polat[a,b], Richard Hill[c,d,e], Teresa W.M. Fan[f], Wolfgang Link[c,d], Marta Cascante[a,b,*]

[a] Department of Biochemistry and Molecular Biology, Faculty of Biology, Universitat de Barcelona, Av Diagonal 643, Barcelona 08028, Spain
[b] Institute of Biomedicine of Universitat de Barcelona (IBUB) and CSIC-Associated Unit, Barcelona, Spain
[c] Centre for Biomedical Research (CBMR), University of Algarve, Campus of Gambelas, Building 8, Room 2.22, 8005-139 Faro, Portugal
[d] Regenerative Medicine Program, Department of Biomedical Sciences and Medicine University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal
[e] Brain Tumour Research Centre, School of Pharmacy and Biomedical Sciences, University of Portsmouth, PO1 2DT, United Kingdom
[f] Department of Toxicology, Markey Cancer Center and Center for Environmental and Systems Biochemistry (CESB), University of Kentucky, Lexington, KY 40536, USA

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ABSTRACT

Selenium supplement has been shown in clinical trials to reduce the risk of different cancers including lung carcinoma. Previous studies reported that the antiproliferative and pro-apoptotic activities of methylseleninic acid (MSA) in cancer cells could be mediated by inhibition of the PI3K pathway. A better understanding of the downstream cellular targets of MSA will provide information on its mechanism of action and will help to optimize its use in combination therapies with PI3K inhibitors. For this study, the effects of MSA on viability, cell cycle, metabolism, apoptosis, protein and mRNA expression, and reactive oxygen species production were analysed in A549 cells. FOXO3a subcellular localization was examined in A549 cells and in stably transfected human osteosarcoma U2foxRELOC cells. Our results demonstrate that MSA induces FOXO3a nuclear translocation in A549 cells and in U2OS cells that stably express GFP-FOXO3a. Interestingly, sodium selenite, another selenium compound, did not induce any significant effects on FOXO3a translocation despite inducing apoptosis. Single strand break of DNA, disruption of tumour cell metabolic adaptations, decrease in ROS production, and cell cycle arrest in G1 accompanied by induction of apoptosis are late events occurring after 24 h of MSA treatment in A549 cells. Our findings suggest that FOXO3a is a relevant mediator of the antiproliferative effects of MSA. This new evidence on the mechanistic action of MSA can open new avenues in exploiting its antitumour properties and in the optimal design of novel combination therapies. We present MSA as a promising chemotherapeutic agent with synergistic antiproliferative effects with cisplatin.

1. Introduction

Lung cancer is a leading cause of cancer-related mortality and has one of the lowest cure rate worldwide [1]. In early stages of the disease, surgery is the common choice while chemotherapy is the main treatment in advanced lung cancer. The search for new synthetic or natural drugs with low systemic toxicity and high efficiency holds great promise to decrease the morbidity and mortality of cancer. The trace element selenium (Se) in various chemical

Abbreviations: MSA, methylseleninic acid; Se, selenium; PPP, pentose phosphate pathway; TCA, tricarboxylic acid; ROS, reactive oxygen species; 1D HSQC, one dimension heteronuclear single quantum coherence; ^{1}H-NMR, proton nuclear magnetic resonance; $^{72h}IC_{50}$, concentration that caused 50% of inhibition of cell growth at 72 h of treatment; FOXO, forkhead box O; FOXM1, forkhead box protein M1; Akt, protein kinase B; PI3K, phosphoinositide-3-kinase; mTOR, mammalian target of rapamycin; CDK, cyclin-dependent kinase; JNK, Jun N-terminal kinase; Bax, B-cell lymphoma-2-associated X protein; PARP, poly(ADP-ribose) polymerase; PRAS40, proline-rich Akt substrate 40 kDa; ERK2, extracellular signal-regulated kinase 2.

* Corresponding author at: Department of Biochemistry and Molecular Biology, Faculty of Biology, Universitat de Barcelona, Av Diagonal 643, Barcelona 08028, Spain. Tel.: +34 93 402 15 93; fax: +34 93 402 15 23.
E-mail addresses: mtarrado@ub.edu (M. Tarrado-Castellarnau), roldancortes@ub.edu (R. Cortés), mzanuy@hotmail.com (M. Zanuy), joseptarrago11@gmail.com (J. Tarragó-Celada), ihapolat@gmail.com (I.H. Polat), drrjhill@gmail.com (R. Hill), twmfan@gmail.com (T.W.M. Fan), walink@ualg.pt (W. Link), martacascante@ub.edu (M. Cascante).
[1] Present address: Almirall R&D Centre, Laureano Miró, 408-410, 08980 Sant Feliu de Llobregat, Barcelona, Spain.

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forms is nutritionally essential for humans but has toxic activity at higher levels [2,3]. To date, the antioxidant and chemopreventive role of different Se agents as a dietary supplement has not been completely elucidated [4]. Se compounds such as sodium selenite (Na_2SeO_3) [5,6] and methylseleninic acid (CH_3SeO_2H, abbreviated as MSA) have also been studied as potential anticancer agents. MSA is a synthetic precursor of methylselenol (CH_3SeH) which induces several cellular, transcriptional and biochemical responses that differ from those induced by selenium forms that are transformed via hydrogen selenide, such as sodium selenite [7,8].

As a constituent of the selenocysteine-containing selenoproteins, selenium has a key role in redox regulation and defence against oxidative stress by greatly enhancing the activity of some antioxidant enzyme systems [9]. Several selenoenzymes, including thioredoxin reductase, iodothyronine deiodinase and glutathione peroxidase, may be associated with cancer development and progression by modulating cell proliferation, transformation, migration and protection against oxidative damage [2]. Selenium deficiency has also been linked to cancer development since it was observed that populations with low selenium intake had greater cancer incidence. Numerous studies and clinical trials have shown that supranutritional doses of individual and mixed selenium compounds inhibit proliferation of cancer cells, induce tumour cell apoptosis, suppress tumour formation and metastasis in animal models and reduce the risk of prostate, lung, breast, and colorectal cancers in humans [9–11]. However, not all selenium compounds have efficacy in chemoprevention, as in a recent large clinical trial (SELECT), selenomethionine was concluded to be ineffective in reducing the risk for prostate cancer development [12].

Using a stable isotope-resolved metabolomic (SIRM) approach, Fan et al. [13] reported that several metabolites, including lactate, glutathione and glutamate are depleted in A549 lung cancer cells by selenite but not by selenomethionine, suggesting multiple perturbations of the central metabolic networks. Interestingly, the reduction in glycolysis, tricarboxylic acid cycle (TCA) and pentose phosphate pathway (PPP) fluxes observed is opposite to those observed when phosphoinositide-3-kinase (PI3K) pathway is activated [14], pointing to the hypothesis that Se agents target this signalling pathway. Among the selenium compounds with anticancer properties, it has been reported that MSA is a potent inhibitor of the growth and survival of human umbilical vein endothelial cells (HUVECs) and that this antiproliferative effect could be enacted through the PI3K pathway [15,16]. Studies with prostate cancer LNCaP, PC-3 (high basal Akt activity) and DU145 cells (low basal Akt activity) have also shown that Akt plays an important role in regulating apoptosis sensitivity to MSA [17]. However, the molecular mechanism of action of MSA is still not fully elucidated.

PI3K/Akt pathway has been shown to be activated in numerous tumours, including lung cancer [18], as it is essential for cell proliferation and survival. Akt is a serine-threonine kinase that is regulated via activation of PI3K. Forkhead box O (FOXO) transcription factors are direct targets of Akt that modulate cellular differentiation, cell cycle, growth, survival, apoptosis, metabolism, DNA repair, resistance to oxidative stress and tumour suppressor pathways [19]. In mammals, FOXO1, FOXO3a and FOXO4 are ubiquitously expressed while FOXO6 is expressed predominantly in neural cells. As transcription factors, FOXO proteins activate or repress the transcription of their target genes through nuclear translocation regulated by post-translational modifications such as phosphorylation, acetylation and ubiquitination [20]. FOXO phosphorylation by Akt impairs its DNA binding activity and promotes its interaction with the chaperone protein 14-3-3, resulting in nuclear exclusion, cytoplasmic accumulation and ubiquitin-proteasome pathway-dependent degradation, thus promoting cell survival. In contrast, FOXO proteins are activated and released from 14-3-3 in the presence of oxidative stress through Jun N-terminal kinase (JNK) signalling [19,21,22]. A hallmark of most cancers where the PI3K pathway is hyperactivated (caused by RAS, PTEN or PI3K mutations) is inactivation of FOXO proteins [23]. In contrast, PI3K depletion results in a significant activation of FOXO transcription factors, induction of apoptosis, decrease of cell viability and G1 cell cycle arrest with inhibition of CDK4/6, cyclin D and accumulation of p27 [24]. Therefore, the search for compounds that promote activation and relocalization of FOXO from the cytoplasm to the nucleus is a promising therapeutic approach for cancer treatment and prevention [25].

In this study, using A549 and U2foxRELOC cells expressing a GFP–FOXO3a fusion protein, we have demonstrated that MSA induces FOXO3a dephosphorylation and nuclear translocation. These findings provide a molecular mechanism to the cytotoxicity, apoptosis, ROS detoxification, cell cycle arrest and metabolic changes observed in A549 cells and implicate FOXO3a as a relevant mediator of MSA antitumour effects.

Moreover, since it has been reported that the antitumour effects of cisplatin are enhanced when it is combined with FOXO nuclear export inhibitors [26–29] and that MSA synergistically sensitized cancer cells in combination with certain chemotherapeutic drugs [30,31], we hypothesized that combined treatment of MSA with cisplatin could be a promising new strategy in cancer therapy.

2. Methods

All products were purchased from Sigma–Aldrich Co. (St Louis, MO, USA), unless otherwise specified.

2.1. Chemicals

MSA was supplied by Dr. Fan (University of Kentucky, KY, USA). Sodium selenite was purchased from Sigma–Aldrich. Stock solutions of 10 mM were prepared with Dulbecco's phosphate buffered saline (PBS). The PI3K inhibitor LY294002 was purchased from Calbiochem (San Diego, CA, USA), antibiotic (10,000 U mL^{-1} penicillin, 10 mg mL^{-1} streptomycin), PBS, Trypsin EDTA solution C (0.05% trypsin –0.02% EDTA) from Biological Industries (Kibbutz Beit Haemet, Israel) and Fetal Bovine Serum (FBS) from Invitrogen (Carlsbad, CA, USA).

2.2. Cell culture

Human lung carcinoma A549 cells (ATCC, Manassas, VA, USA) were grown in RPMI-1640 medium with L-glutamine and 10 mM D-glucose prepared following the manufacturer's instructions. Human osteosarcoma stably transfected U2foxRELOC cells (a gift from Dr. Wolfgang Link), human osteosarcoma U2OS cells, human large cell lung cancer NCI-H460 cells, human ovary adenocarcinoma OVCAR3 cells, human embryonic kidney 293 (HEK293) cells and adipocyte-like differentiated 3T3-L1 cells (ATCC) were grown in DMEM with L-glutamine and 25 mM D-glucose. Human colorectal carcinoma HCT116 cells (ATCC) were cultured in DMEM:HAM F12 (1:1) with L-glutamine and 12.5 mM D-glucose. Human breast adenocarcinoma MCF7 cells (ATCC) were cultured in MEM medium without phenol red (Gibco, Thermo Fisher Scientific Inc., Waltham, MA, USA) containing 10 mM D-glucose, 2 mM L-glutamine, 1 mM pyruvate (Biological Industries), 0.01 mg mL^{-1} insulin and 1% non-essential aminoacids (Biological Industries). Media were supplemented with 10% heat-inactivated FBS, penicillin (50 U mL^{-1}) and streptomycin (50 μg mL^{-1}). U2foxRELOC cells, which express a resistance to Geneticin, were incubated with G418 (Gibco) at 100 μg mL^{-1}. 3T3-L1 pre-adipocyte cells were grown in DMEM