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L-Methionine inhibits growth of human pancreatic cancer cells

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Abstract

We have previously shown that L-methionine inhibits proliferation of breast, prostate, and colon cancer cells. This study extends these findings to BXPC-3 (mutated p53) and HPAC (wild-type p53) pancreatic cancer cells and explores the reversibility of these effects. Cells were exposed to L-methionine (5 mg/ml) for 7 days or for 3 days, followed by 4 days of culture without L-methionine (recovery). Cell proliferation, apoptosis, and cell cycle effects were assessed by flow cytometry after staining for Ki-67 or annexin V/propidium iodide. Cell proliferation was reduced by 31–35% after 7 days of methionine exposure; the effect persisted in BXPC-3 and HPAC cells after 4 days of recovery. Methionine increased apoptosis by 40–75% in HPAC cells, but not in BXPC-3 cells. Continuous exposure to methionine caused accumulation of BXPC-3 cells in the S phase and HPAC cells in both the G0/G1 and S phases; however, after 4 days of recovery, these effects disappeared. In conclusion, L-methionine inhibits proliferation and interferes with the cell cycle of BXPC-3 and HPAC pancreatic cancer cells; the effects on apoptosis remarkably persisted after methionine withdrawal. Apoptosis was induced only in BXPC-3 cells. Some of the differences in the effects of methionine between cell lines may be related to disparate p53 status. These findings warrant further studies on the potential therapeutic benefit of L-methionine against pancreatic cancer.

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Conflicts of interest

There are no conflicts of interest.

Keywords

BXPC-3 cells; HPAC cells; methionine; p53; pancreatic cancer cells

Introduction

The efficacy of conventional chemotherapy for treatment of patients with advanced cancer, including pancreatic cancer, is limited, indicating the need to identify new therapeutic strategies. Recombinant proteins have been increasingly used for therapies in the recent years [1], possessing a variety of potential clinical benefits for some major categories of diseases, including cancer [2,3]. We have previously shown that L-methionine possesses strongly inhibitory effects on proliferation of cancer cells that are associated with post-translational modification of the tumor suppressor p53 [4]. Further, we have demonstrated that methionine treatment selectively inhibits cell proliferation and cell cycle progression of breast (MCF-7) and prostate cancer (LNCaP) cells, but has no significantly inhibitory effect on immortalized but nontumorigenic human breast and prostate epithelial cells [5]. Methionine also induces common changes in molecular signatures of breast cancer cells and prostate cancer cells [6]. These results suggest an anticancer potential of this important amino acid and point to the possibility of developing methionine analogs as new therapeutic agents.

Methionine is an essential amino acid that plays fundamental roles in protein synthesis and a number of other biochemical and cellular processes [7–11]. In addition, methionine acts as a precursor of glutathione, an important tripeptide that reduces reactive oxygen species, thereby protecting cells from oxidative stress [12]. It is also involved in DNA and protein methylation by serving as the methyl-group donor, consequently playing a role in regulation of gene expression and protein functions [13]. Further, methionine is required for the biosynthesis of the polyamines spermine and spermidine, which are critically involved in a number of molecular biology activities, including cell proliferation [14], whereas certain methionine analogs are known to be capable of inhibiting protein synthesis [15,16].

The present study was designed to determine the effects of L-methionine on pancreatic cancer cells and to explore the mechanisms that underlie methionine-mediated inhibitory effects on cell survival and cell cycle progression, as well as their reversibility.

Materials and methods

Cell lines and culture conditions

Wild-type p53-expressing HPAC pancreatic cancer cells [17] and BXPC-3 pancreatic cancer cells [18], containing inactive (mutated) p53, were obtained from the American Type Culture Collection (Manassas, Virginia, USA). BXPC-3 cells were grown in RPMI 1640 (Mediatech-Cellgro, Herndon, Virginia, USA) with 10% fetal bovine serum (HyClone, Logan, Utah, USA) and HPAC cells in DMEM/F-12 (1 : 1) with 10% fetal bovine serum. L-Methionine (analytical grade; Sigma M5308) was obtained from Sigma (St Louis, Missouri, USA) and incorporated in the respective media at a final concentration 5.0 mg/ml.

Experimental design

The in-vitro effects of L-methionine were evaluated for 7 days; there were two treatments: complete treatment, 5 mg/ml of L-methionine for 7 days, and incomplete treatment, 5 mg/ml of L-methionine for 3 days plus 4 days in culture media without L-methionine. All experiments were carried out in triplicate.

Assessment of cell proliferation

The cells were harvested, counted, and stained for Ki-67 according to the manufacturer's standard protocol (BD Via-Probe Cell Viability Assay; BD Biosciences, San Jose, California, USA). After adding PE-conjugated antibody and 20 µl of the BD Via-Probe Cell Viability Solution, the samples were analyzed by flow cytometry (FACSCanto; Becton Dickson, Franklin Lakes, New Jersey, USA).

Cell death assay

A total of 2×10^6 cells were collected and stained with annexin V-fluorescein isothiocyanate according to the manufacturer's protocol (Invitrogen, Grand Island, New York, USA) and propidium iodide, and the samples were analyzed by flow cytometry.

Cell cycle analysis

Adherent and nonadherent cells were harvested and incubated with RNase A (10 mg/ml; Invitrogen Life Technologies, Van Allen, Carlsbad, California, USA) and 164 µl/tube of hypotonic fluorescent solution. A total of 100 000 cells were analyzed by flow cytometry after staining with propidium iodide, and the resulting data were analyzed using FlowJo software 7.6.1 (Tree Star, Ashland, Oregon, USA).

Statistics

The results are expressed as mean±SD and were analyzed by one-way analysis of variance (with post-hoc Tukey's analysis); all treatments were compared with each other using Graph Pad Prism 5 software, considering *P*-values less than 0.05 to be statistically significant.

Results

Effects of L-methionine on cell proliferation

We determined the effects of 5 mg/ml L-methionine on cell growth (Ki-67) of BXPC-3 and HPAC pancreatic cancer cells after 7 days of incubation with methionine (complete treatment) or 3 days of incubation with methionine followed by 4 days of recovery by culturing in media without methionine (incomplete treatment). Both complete and incomplete treatment with L-methionine significantly reduced growth of BXPC-3 cells by 31 and 32%, respectively ($P < 0.001$). Growth of HPAC cells was also significantly reduced (by 35%; $P < 0.001$) following complete treatment with L-methionine, but incomplete treatment reduced growth by only 18% ($P < 0.05$) (Table 1 and Supplemental digital content 1, <http://links.lww.com/ACD/A44>).

Effects of L-methionine on cell cycle progression

Effects of L-methionine on cell cycle progression were evaluated by flow cytometry. BXPC-3 cells treated with L-methionine for 7 days accumulated in S phase ($P < 0.01$), suggestive of a reduced S to G2 transition. In contrast, HPAC cells accumulated in both S and G0/ G1 phases ($P < 0.001$ and < 0.01 , respectively), suggesting an interference with both G1–S and S–G2 transitions. The cell cycle profile after incomplete L-methionine treatment was similar to that of the controls for BXPC-3 cells, but HPAC cells were still accumulating in the G0/G1 phase (Table 2).

Effects of L-methionine on cell death

BXPC-3 and HPAC cancer cells differed greatly in the effects of L-methionine on early apoptosis (annexin V-positive cells) and late apoptosis (annexin V-positive and propidium iodide-positive cells), as assessed by flow cytometry. Apoptosis of BXPC-3 cells was not affected significantly by complete L-methionine treatment, but was reduced by 35% following recovery (incomplete treatment; $P < 0.05$). In contrast, apoptosis (only late apoptosis) of HPAC cells was increased by 76% after complete treatment ($P < 0.05$) and by 43% after incomplete treatment (not statistically significant) (Table 3).

Discussion

Methionine is an essential amino acid that plays a major role in protein and DNA synthesis and 1-carbon metabolism, and it contributes to the cellular pool of organic sulfur. In our previous studies [4–6], we observed that methionine had inhibitory effects on cell proliferation in both prostate and breast cancer cell lines that contain wild-type p53. We hypothesized that methionine would also inhibit growth of other cancers cells such as pancreatic cancer cells. Further, we reasoned that if methionine acted through modulation of p53, a decrease in cellular growth might be observed in cells expressing functionally active p53, but not in cells with inactive mutant p53 or in which the expression of p53 was reduced or absent.

In the present study, we tested these hypotheses. We explored the effects of methionine on proliferation of pancreatic cancer cells, comparing HPAC cells expressing wild-type p53 with BXPC-3 cells expressing mutant p53. Using a Ki-67 expression assay, we found that (a) L-methionine (5 mg/ml) inhibited the growth of both HPAC and BXPC-3 cell lines after complete treatment for 7 days and (b) these inhibitory effects were partially sustained when the treatment was suspended after 3 days and the cells were allowed to recover for 4 days (incomplete treatment). The HPAC and BXPC-3 cells were more sensitive to methionine-mediated inhibitory effects on cell proliferation than were MCF-7 cells [4,5]. The number of HPAC cells undergoing apoptosis was increased by L-methionine treatment, whereas apoptosis was reduced in BXPC-3 cells, even after a 3 day recovery period (incomplete treatment). These marked differences may also be related to the different p53 statuses of the HPAC and BXPC-3 cells (mutant and wild type, respectively). However, we previously found that L-methionine increased apoptosis in MCF-7 cells, but hardly affected apoptosis in LNCaP cells, although both cell lines contained wild-type p53 [5]. Thus, we found support for the hypothesis that methionine also inhibits growth of pancreatic cancer cells, but p53

mutational status did not appear to consistently affect the inhibitory effects of methionine on proliferation and apoptosis of these and other cancer cells.

Cell cycle progression, in contrast, was differentially affected by methionine treatment in cells with different p53 status. Methionine inhibited proliferation of HPAC cells (wild-type p53) by causing accumulation of cells in the G1 phase, whereas it inhibited proliferation of BXPc-3 cells (mutant p53) by causing accumulation of cells in the S phase. We previously obtained similar results on comparing the effects of methionine on cell cycle progression of LNCaP (wild-type p53) and DU-145 (inactive mutant p53) prostate cancer cells [5]. Consistent with these observations, the results of published genomic studies indicate that p53 induces or inhibits the expression of more than 150 genes that arrest mammalian cells either in the S-to-G1 phase transition or in the G2 phase before mitosis [19]. This arrest in cell cycle progression is thought to provide p53 with time to modulate repair of DNA damage [20,21], whereas in some circumstances cell cycle arrest is permanent and indistinguishable from senescence [22,23]. The growth-inhibitory effects of methionine are conceivably mediated by *S*-adenosylmethionine, a derivative of methionine and a methyl donor for biological transmethylation reactions [8]. *S*-Adenosylmethionine is capable of inducing growth arrest through the DNA damage-inducible gene 45 β (*GADD45* β) in the wild-type p53-containing human hepatoma cell line, HepG2, but not in the p53-null cell line, Hep3B [24].

Collectively, our observations suggest that some of the effects of methionine on proliferation and cell death of cancer cells may be modulated by the p53 status of the cells (wild type vs. mutant). These data extend and provide additional support to some of our previous findings with other cancer cell lines that suggest that p53 status is a key player in mediating the inhibitory effects of methionine on cell cycle progression of cancer cells. However, our current results suggest more complexity in the mechanisms of methionine-induced inhibition of cancer cell growth, because the apoptosis-inducing and some antiproliferative effects of methionine may not be related to the p53 status of cancers cells. Other mechanisms of action also need to be considered, particularly effects on DNA methylation and proapoptotic signaling pathways. Future studies should focus on these possible mechanisms by examining which apoptosis events are involved and whether other signaling pathways or DNA methylation is affected. The observed effectiveness of a short course of methionine exposure in causing a sustained inhibitory effect on proliferation of pancreatic cancer cells is remarkable. We have also observed this sustained effect in MCF-7 cells (unpublished data). This finding suggests that this amino acid can have lasting effects, possibly through effects on methylation of critical genes, which merits further investigation. The observed sustained growth-inhibitory effects suggest that analogs of methionine may have therapeutic potential for a variety of malignancies, provided that they lack the potential negative effects of methionine itself, such as the well-known methionine dependence of many tumor cells [25]. Given that chemotherapies for treatment of pancreatic cancer are not very effective, the growth-inhibitory effects of L-methionine on pancreatic cancer cells suggest that methionine and its analogs might particularly have therapeutic potential for pancreatic cancer, indicating that further mechanistic studies are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1Effect of exposure to L-methionine on cell proliferation of BXP3 and HPAC pancreatic cancer cells^a

Cell line	Control	Complete treatment	Incomplete treatment
BXPC-3	99.2±0.53	68.5±1.05**	67.4±2.00**
HPAC	97.0±0.14	63.5±5.11**	79.2±1.01*†

^aData are presented as mean±SD of % Ki-67-positive cells in three separate experiments.* $P < 0.05$.** $P < 0.001$ for difference with control value.† $P < 0.01$ for difference with complete treatment.

Table 2

Effects of 24-h exposure to L-methionine on cell cycle distribution of BXP3 and HPAC pancreatic cancer cells^a

	Control			Complete treatment			Incomplete treatment		
	G0/G1	S	G2/M	G0/G1	S	G2/M	G0/G1	S	G2/M
BXPC-3	63.2±0.01	4.3±0.27	32.6±0.26	67.8±2.26	14.5±3.43 [†]	17.7±1.17	61.1±1.35 [‡]	1.9±2.02 ^{§#}	36.0±1.91
HPAC	58.2±0.21	8.4±2.46	29.6±7.88	76.7±2.26 [§]	17.8±1.83 [†]	8.0±2.39 [†]	70.5±1.21 ^{§¶}	4.0±1.99 [#]	25.5±7.15 ^{//}

^aData are presented as mean±SD of % of cells in the G0/G1, S, and G2/M phases of the cell cycle in three separate experiments.

[†] $P < 0.05$.

[§] $P < 0.001$ for difference with control value.

^{//} $P < 0.05$.

[¶] $P < 0.01$.

[#] $P < 0.001$ for difference with complete treatment.

Table 3

Effect of exposure of BXP3 and HPAC pancreatic cancer cells to L-methionine on early and late apoptosis (annexin V-positive and annexin V/propidium iodide-positive cells, respectively)^a

Cell line	Control		Complete treatment		Incomplete treatment	
	Ann. V+	Ann. V + /PI +	Ann. V+	Ann. V + /PI +	Ann. V+	Ann. V + /PI +
BXPC-3	4.3±0.4	10.0±0.2	4.0±0.3	7.9±1.4	3.0±0.3 ^{*†}	6.5±1.7 [*]
HPAC	3.7±0.5	14.9±1.4	3.5±1.8	26.2±5.6	2.5±2.2	21.3±18.3

Ann. V + , annexin V positive; PI + , propidium iodide positive.

^aData are presented as mean±SD of % of positively stained cells in three separate experiments.

^{*} $P < 0.05$.

[†] $P < 0.001$ for difference with control value.