Selenomethionine induces sustained ERK phosphorylation leading to cell-cycle arrest in human colon cancer cells

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Selenomethionine (SeMet) is being tested alone and in combination with other agents in cancer chemoprevention trials. However, the molecular targets and the signaling mechanism underlying the anticancer effect of this compound are not completely clear. Here, we provide evidence that SeMet can induce cell-growth arrest and that the growth inhibition is associated with S-G2/M cell-cycle arrest. Coincidentally with the cell-cycle arrest, we observed a striking increase in cyclin B as well as phosphorylation of the cyclin-dependent kinase Cdc2. Since activation of the mitogen-activated protein kinase (MAPK) cascade has been associated with cell-cycle arrest and growth inhibition, we evaluated the activation of extracellular signal-regulated kinase (ERK). We found that SeMet induced phosphorylation of the MAPK ERK in a dose-dependent manner. We also demonstrate phosphorylation of ribosomal S6 kinase (p90RSK) by SeMet. Additionally, we show phosphorylation of histone H3 in a concentration-dependent manner. Furthermore, the phosphorylation of p90RSK and histone H3 were both antagonized by the MEK inhibitor U0126, implying that SeMet-induced phosphorylation of p90RSK and histone H3 are at least in part ERK pathway dependent. Based on these results, we propose that SeMet induced growth arrest and phosphorylation of histone H3 are mediated by persistent ERK and p90RSK activation. These new data provide valuable insights into the biological effects of SeMet at clinically relevant concentrations.

Introduction

Currently, high selenium containing yeast is being investigated in a phase II double-blind, placebo-controlled, trial designed to assess the effect of selenium and celecoxib, either individually or in combination, on colon polyp recurrence (1). The launching of this trial is largely driven by the seminal findings of Clark et al. (2) that high selenium containing yeast supplementation was capable of significantly reducing the incidence of colon (RR = 0.42), lung (RR = 0.54) and prostate cancers (RR = 0.37). The colon prevention trials with selenium provide the establishment of a repository of colon biopsy tissue and hematopoietic cell types. These materials are archived for research discoveries in the future. One of the secondary objectives of this trial is to study the cellular and molecular biomarkers using the banked samples and to elucidate their relevance with respect to colon carcinogenesis and drug effects. Despite the considerable public interest in the potential benefit of selenium chemoprevention of colon cancer, little information is currently available on the molecular targets or the signaling mechanisms underlying the anticancer effects of high selenium containing yeast. Our present study was aimed at addressing this gap of knowledge with the use of human colon cancer cell lines.

Analytical speciation studies showed that the bulk of the selenium in selenized-yeast is in the form of selenomethionine (SeMet) (85%) (3). Thus, the anticancer effects of high selenium containing yeast may be due in large part to SeMet. SeMet represents an organic form of selenium and is more bioavailable than an inorganic form such as Na2SeO3 (4). Substitution of methionine by SeMet represents a mechanism for non-specific incorporation of selenium into proteins in vivo. SeMet is metabolized primarily in the liver to a monomethylated intermediate for the expression of its anticancer activity and epithelial tissues typically retain a low capacity to generate a mono-methylated Se-metabolite form SeMet (5–9). Consequently concentrations of SeMet that are 20–100 times above the physiological levels have been used in cultured cells not relevant to physiological concentrations of selenium. In the present study, we developed a low-dose multitreatment protocol using SeMet within the physiological range of selenium in the circulation and tissues.

Although extracellular signal-regulated kinase (ERK) is thought to play a key role in the proliferative process, recent studies suggest that persistent activation of ERK might mediate cell-cycle arrest and differentiation (10,11). After activation, phospho-ERK is translocated from the cytoplasm to the nucleus, where it can phosphorylate and activate multiple nuclear substrates such as transcription factors and other kinases leading to altered gene expression. More recently, it has become clear that chromatin structure plays an important role in eukaryotic gene regulation. An increasing body of evidence indicates that the MAP kinase cascades direct the phosphorylation of upstream transcription factors and co-activators controlling intermediate early genes. They also act directly on chromatin proteins such as histone H3 and the high-mobility group protein HMG-14 to modify chromatin concomitant with gene induction (12–17).

In this study, we first examine the dose-dependent effect of SeMet on the growth of HCT116 and SW48 human colon cancer cells. We then show that growth inhibition by SeMet is probably attributable to an effect on the cell cycle. We also demonstrate alterations in the expression of the S-G2/M cyclins concomitant with SeMet induced cell-cycle effects. Next, we examine the activation of ERK pathway to gain further insight into the signaling pathways that may play a role in the regulation of these cellular events. Finally,

Abbreviations: ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; RSK, ribosomal S6 kinase; SeMet, l-selenomethionine; SRB, sulforhodamine B.
we report that phosphorylation of histone H3 is associated with increased treatment with SeMet, which might further lead to nucleosomal modifications. From these findings, we are able to develop a model of the signal events that might explain the action of SeMet in blocking cell-cycle progression in colon cancer cells.

Materials and methods

Cell culture

The DNA mismatch repair deficient human colorectal carcinoma cell lines, HCT116 and SW48 were both obtained from the American Type Culture Collection (Rockville, MD). HCT116 has been reported to have a transversion in the mismatch repair gene hMLH1, producing a premature stop codon (18-20), SW48 lacks MLH1 expression because of hypermethylation of the hMLH1 gene promoter (21). HCT116 cells were maintained in monolayer culture in McCoy’s 5A media and SW48 in RPMI 1640 (Cellgro Mediatech, Herndon, VA), supplemented with 10% fetal bovine serum (FBS; Omega Scientific, Tarzana, CA), 2 mM L-glutamine, 50 IU/ml penicillin and 50 μg/ml streptomycin (Gibco/Invitrogen, Carlsbad, CA) in a humidified incubator with 95% air and 5% CO2 at 37°C.

Multi-treatment protocol

Cells in the log phase were harvested by trypsinization and plated at a density of 2 × 10⁴ cells/plate (HCT116) or 4 × 10⁴ cells/plate (SW48) in 100 mm tissue culture plates. Cells were allowed to adhere to the plates for 24 h before treatment. Thereafter cells were treated with 5-22 μM of SeMet (Sigma). Cells were treated for up to 8 or 12 days. Once treated, cells were either collected (removed by trypsinization) or re-treated by adding fresh media and fresh SeMet every 2 days out to day 8 or to day 12. The rationale for using a multi-treatment protocol was to investigate a low concentration of SeMet every 2 days out to day 8 or to day 12. The rationale for using isoosmolar followed by an ethanol wash. The resulting RNA product was re-dissolved in RNAse free water. Analysis of cyclin expression was performed using a commercially available RNase protection assay. An hyc-c1 probe (BD PharMingen, San Diego, CA) was synthesized using the protocol described by the manufacturer. Probe labeled with [α-32-P]UTP (ICN, Irvine, CA) was allowed to hybridize 5-10 μg RNA overnight. The hybridized RNA was subjected to RNase treatment, purified and protected RNA fragments were resolved on a 6% acrylamide gel for 10 min at 1200 V, dried for 1 h at 80°C without vacuum. Cyclin RNA levels were analyzed by autoradiography and compared with internal standard L32 expression.

Protein extraction and western blotting

Treated and control cells were washed with cold 1× PBS, lysed and sonicated in cold RIPA buffer [10 mM Tris⋅HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1% v/v Nonidet P-40, 1% sodium deoxycholate, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride and protease inhibitor cocktail (Sigma)] on ice for 30 min. Lysates were cleared by centrifugation at 13 000 g for 30 min at 4°C. Equal amounts of cell extracts (50 μg) (bicinechonic acid assay, BCA, Pierce, Rockford, IL) were resolved on SDS-polyacrylamide gels and then transferred onto a polyvinylidene difluoride membranes (Bio-Rad Laboratories, Hercules, CA). After blocking membranes in 5% non-fat dry milk, they were probed overnight with the primary antibody. Membranes were then washed three times in 1× PBS containing 0.5% Tween 20 (PBS-T) for 20 min before being incubated with the secondary horseradish peroxidase-linked antibody diluted in 1% bovine serum albumin PBS-T for 1 h. Detection was performed using an enhanced chemiluminescence detection system (Amersham Biosciences, Piscataway, NJ). Relative protein blot intensities were determined using one-dimensional Image Analysis Software (Kodak). Graphs were constructed using the sum intensity representing the sum of all the pixel intensities in the band rectangle.

Real-time PCR

SeMet (22 μM) treated and non-treated HCT116 cells were collected at day 8 and total RNA was isolated. To limit the possibility of detection of genomic DNA, total RNA was subjected to DNase treatment. The cDNA was then produced by omniscrypt reverse transcriptase (Qiagen, Valencia, CA) and quantified using Taqman Universal Master Mix (Applied Biosystems, Foster City, CA) on a Gene Amp 5700 sequence detector (Applied Biosystem). Primers and probes for cdc2 (B-4) Ser-383, were designed by Applied Biosystem. The resulting RNA product was then subjected to RNase treatment, purified and protected RNA fragments were resolved on a 6% acrylamide gel for 10 min at 1200 V, dried for 1 h at 80°C without vacuum. Cyclin RNA levels were analyzed by autoradiography and compared with internal standard L32 expression.

RNase protection assay

Cells were homogenized using Trizol reagent (Gibco/Invitrogen) as described by the manufacturer and as carried out previously by our group (25). RNA was separated using Trizol and chloroform. Precipitation was achieved using isopropryl alcohol followed by an ethanol wash. The resulting RNA product was re-dissolved in RNAse free water. Analysis of cyclin expression was performed using a commercially available RNase protection assay. An hyc-c1 probe (BD PharMingen, San Diego, CA) was synthesized using the protocol described by the manufacturer. Probe labeled with [α-32-P]UTP (ICN, Irvine, CA) was allowed to hybridize 5-10 μg RNA overnight. The hybridized RNA was subjected to RNase treatment, purified and protected RNA fragments were resolved on a 6% acrylamide gel for 10 min at 1200 V, dried for 1 h at 80°C without vacuum. Cyclin RNA levels were analyzed by autoradiography and compared with internal standard L32 expression.

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Statistical analyses

All experiments were repeated on at least three separate occasions. Values were reported as means ± SD. Differences between means were compared using the Student’s t-test and P values of <0.05 were considered significant.
Results

SeMet effects on cell growth and apoptosis
To understand how SeMet and, therefore, high selenized yeast may be acting as an anti-proliferative agent, a repeated low-dose model was established to mimic more closely the dosing of patients (Figure 1). Cell counting was done to quantify any growth effect caused by SeMet. We observed a time-dependent growth inhibition in both HCT116 and SW48 cells treated with SeMet at 22 μM (Figure 2). The effects of SeMet on the growth of these tumor cells were obvious at days 6 and 8. HCT116 cells (Figure 2A) exhibit an inhibition of cell growth at day 6 with a 43% decrease ($P = 1.12$, E-05) and at day 8 with a 38% decrease ($P = 6.35$, E-04) in the number of treated cells compared with control cells. The effect of SeMet on SW48 (Figure 2B) was also significant with a decrease in growth inhibition of 51% at day 6 ($P = 1.47$, E-03) and of 77% at day 8 ($P = 2.55$, E-06). To further investigate the role of SeMet in cell growth inhibition, cell death was evaluated by 7 amino-actinomycin D (7-AAD) staining. There was no difference in the number of cells undergoing apoptosis between control and SeMet-treated HCT116 and SW48 cells (data not shown). These findings were confirmed by morphological analysis (data not shown). Thus, increased apoptosis cannot account for the growth inhibitory effects of SeMet in HCT116 and SW48 colon cancer cells at 22 μM.

Cell-cycle arrest induced by SeMet
To explore the role of SeMet in the cell cycle, HCT116 cells were treated with 22 μM SeMet for up to 8 days and then analyzed for cell-cycle alterations by flow cytometry (Figure 3). We observed a decrease in the percentage of cells

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**Fig. 1.** Design of the 12-day *in vitro* repeated dosing experiments for administering SeMet to colon cancer cells.

**Fig. 2.** The effects of SeMet on colon cancer cell growth. Growth curve for HCT116 cells (A) and for SW48 cells (B) treated with 22 μM SeMet. Colon cancer cell lines were treated with SeMet (square) or non-treated, control (triangle) for up to 8 days as described under multi-treatment protocol in the Materials and methods. Cell number was enumerated using trypan blue exclusion. Mean ± SD ($n = 3$).

**Fig. 3.** The cell-cycle distribution of colon cancer cells treated with SeMet. HCT116 cells were treated with SeMet for the indicated time points and stained with propidium iodide followed by flow cytometry analysis. Results are expressed as percentage of cells in $G_0/G_1$, $S$ and $G_2/M$ phases of the cell cycle. The data are representative of three independent experiments.
in G0/G1 treated with SeMet at days 6 and 8. Whereas HCT116 cells demonstrated an increase in the number of cells in the S-G2/M phase of the cell cycle when treated with SeMet, with respect to S-phase, HCT116 controls at days 6 and 8 had 16 and 10% of cells in S phase. In comparison, SeMet-treated cells had almost double with 34 and 17%, respectively. With regard to mitosis, the vehicle-control cells had 8 and 5% at days 6 and 8, respectively, whereas SeMet treatment had 12 and 10% in the G2/M phase of the cell cycle. We observed a similar effect on the cell cycle in SW48 cells. However, this effect was not as obvious compared with HCT116 cells (data not shown).

**SeMet affects cyclin RNA and protein expression**

RNase protection assays were conducted to analyze cells for alterations in cyclin RNA expression that may explain the observed growth inhibition and S-G2/M arrest. RNA levels for both cyclin A and cyclin B were elevated in SeMet-treated cells compared with control cells (Figure 4A). Elevated cyclin RNA levels were seen in SeMet-treated HCT116 cells both at days 6 and 8.

To evaluate cyclins at the protein level, we performed western blot analysis on SeMet-treated cells. We observed only a slight increase in cyclin A protein levels at the time points tested, whereas there was an obvious increase in cyclin B protein levels in SeMet treated cells at day 8 (Figure 4B).

**Phosphorylation of Cdc2 on Tyr-15 coincides with S-G2/M cell-cycle arrest**

The phosphorylation of Cdc2 on Tyr-15 causes cells to arrest in the G2/M transition phase of the cell cycle (27,28). To determine if the accumulation of cells in the S-G2/M

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**Fig. 4.** The effects of SeMet on cell cycle regulators. (A) Cyclin RNA levels using RNase protection assay. Representative phosphorimage of an assay in HCT116 cells treated with SeMet at the indicated time points. Note increased cyclin A and cyclin B RNA levels at days 6 and 8 compared with vehicle control cells. (B) Cyclin protein expression of HCT116 cells treated with SeMet using western blot analysis. HCT116 cells were treated with SeMet for up to 8 days. Cell extracts were prepared at the indicated time points, and cyclin A, cyclin B1 were detected by immunoblotting. Uniform loading of lysates was confirmed by immunoblotting for α-tubulin. (C) The effects of SeMet on Cdc2 phosphorylation. HCT116 cells were treated with SeMet for up to 8 days. Cell extracts were prepared at the indicated time points and immunoblotted with anti-phospho Cdc2 Tyr-15 and anti-Cdc2 antibodies at the indicated time points. Equal loading was determined by anti-α-tubulin expression analysis. (D) The effects of SeMet on Wee1 kinase. HCT116 cells were treated with SeMet 22 μM for up to 8 days. RNA was then isolated and reverse transcribed. The resulting cDNA was then subjected to real-time PCR using Wee1 primers and probe. GAPDH was used to account for loading differences.
phase observed by flow cytometry was associated with phosphorylation of Cdc2, we examined the phosphorylation status of Cdc2 at Tyr-15 by western analysis. After treatment with 22 μM SeMet we observed an increase in Cdc2 phosphorylation (taken at days 6 and 8) compared with control cells (Figure 4C, upper panel). There were no obvious changes in non-phosphorylated Cdc2 levels (Figure 4C, third panel).

**Wee1 kinase level is increased by SeMet**

Cdc2 is heavily regulated through phosphorylation. Its activity is suppressed through phosphorylation on Tyr-15 by Wee1 kinase. We investigated the role of Wee1 in SeMet-treated HCT116 cells and found that the RNA level of Wee1 measured by real-time PCR was significantly increased (P < 0.05) at day 8 (Figure 4D). This finding suggests a potential role of Wee1 kinase in SeMet treatment.

**Effects of SeMet on the mitogen-activated protein kinase (MAPK) pathway**

To evaluate the effect of SeMet in HCT116 in greater detail, we assessed the impact on the MAPK signaling pathways. Activation of both p38 and ERK has been associated with G2/M arrest (10,29). We evaluated the phosphorylation status of JNK, p38 and ERK. We found no differences between control and SeMet-treated cells with respect to JNK and p38 phosphorylation status (data not shown). However, there were differences in ERK activation. Treatment with SeMet increased ERK phosphorylation on Tyr-204 in HCT116 at days 6 and 8 (Figure 5A, upper panel). Densitometric scan of p-ERK normalized with α-tubulin showed an increase of p-ERK1 p44 (a) and p-ERK2 p42 (b) with SeMet at both days (Figure 5C). In contrast, no change in total ERK (p42/p44) was observed during SeMet stimulation at days 6 and 8 in HCT116 cells (Figure 5B). Pharmacological treatment of HCT116 with the MEK1/2 inhibitor U0126 (10 μM) for 1 h at day 8 decreased the phosphorylation of ERK induced by SeMet in HCT116 (Figure 5D). The decrease in phosphorylation was obtained in the presence and in the absence of FBS. Moreover, U0126 had no effect on the expression of ERK1 and ERK2 demonstrating the specificity of the inhibitor on phosphorylation (Figure 5E). Taken together, the data support the notion that G2/M arrest of tumor cells after SeMet treatment involves activation of the MAP kinase ERK.

**SeMet-induced ERK activation is related to p90RSK phosphorylation**

The p90RSK family of serine/threonine kinases are known to be activated by ERK in response to stimuli (11). Thus, the fact that SeMet induced ERK activation prompted us to examine whether p90RSK phosphorylation was involved in SeMet actions. We compared the effects of SeMet on RSK1/2 phosphorylation at serine residue 380 because the phosphorylation at Ser-380 residue is thought to be related to ERK kinase activity (30). We found that SeMet stimulated RSK phosphorylation at Ser-380 at days 6 and 8 (Figure 6A). To examine the relationship between RSK and ERK phosphorylation, we treated cells with U0126 to determine whether this MEK inhibitor could reverse the effect of SeMet on RSK phosphorylation. Figure 6B shows that U0126 effectively antagonized SeMet-mediated RSK phosphorylation, suggesting that persistent ERK activation by SeMet leads to RSK phosphorylation and activation. Although MSK1/2 are also downstream of ERK (31), we did not observe phosphorylation of MSK by SeMet in these experiments (data not shown).
Histone H3 is phosphorylated in SeMet-treated cells

Previous reports have suggested roles for both MSK and RSK in histone phosphorylation downstream of MAP kinases (14,15,32). Histone H3 is highly phosphorylated on condensed chromosomes during mitosis (14,16,17). The presence of Ser-10 histone H3 phosphorylation is considered an excellent mitosis-specific marker and is also indicative of cell-cycle arrest. Our experiments showed that SeMet induces an increase in the phosphorylation state of histone H3 on Ser-10 after 6 and 8 days of consecutive treatment in both HCT116 cells and SW48 cells (Figure 7A). The MEK inhibitor U0126 reduces the phosphorylation state of histone H3 in SeMet-treated HCT116 cells (Figure 7B). This suggests that SeMet induced ERK activation can lead to histone H3 phosphorylation. No phosphorylation of Elk-1 on Ser-383, which was another possible effector of ERK, was observed (data not shown).

SeMet induces ERK, RSK and histone H3 phosphorylation at physiological concentrations

We first investigated the ability of SeMet to inhibit HCT116 cell growth by the SRB assay (7 days) at a concentration range between 5 and 22 μM. We found that a single dose of SeMet inhibited tumor cell growth in a dose-dependent manner, being significant at 10 μM (P = 0.03) and 22 μM (P = 0.02) (Figure 8A). Next, we examined the extent of ERK phosphorylation in cells treated up to 12 days with 5, 10 or 22 μM SeMet. We found that SeMet caused a concentration-dependent activation of ERK (Figure 8B, upper panel). We also observed phosphorylation of RSK and histone H3 in low-dose SeMet-treated cells (Figure 8B).
Discussion

Colorectal cancer remains the second leading cause of cancer mortality in the USA (33). Risk factors include a combination of genetic and environmental (nutrition and physical activity) variables. Surveillance, early detection and prevention are central to the reduction of incidence and mortality from this disease (34). Although current colorectal screening methods such as fecal occult blood testing and screening colonoscopy are known to reduce mortality from this disease, patient compliance with screening recommendations remains low (35–38). In patients that comply, the removal of precursor lesions (adenomas) can reduce the incidence of subsequent colorectal cancer (39,40). However, the colonoscopic polypectomy has an associated miss-rate (41), and the polyv recurrence rate is often high (41). Thus, there is considerable need to develop dietary and chemopreventive intervention strategies that can be applied to large populations to reduce adenoma recurrence and colorectal cancer risk.

In human clinical trials, selenium, in the form of high selenium containing yeast has been shown in secondary analysis of a phase II study to reduce the incidence and mortality from colon cancer, and experiments in laboratory animals have demonstrated that various selenium compounds inhibit colorectal carcinogenesis (2). Recently a multi-center phase II double-blind, randomized, factorial trial to determine the efficacy of celecoxib and selenium supplementation in the form of high selenium containing baker’s yeast separately and combined in reducing colorectal adenoma recurrence in patients with sporadic has been undertaken (1). SeMet is the major selenium compound in the selenized yeast (3). However, the mechanism of action of SeMet remains obscure. Furthermore, the optimal concentration of selenium supplementation and exactly what groups will benefit from selenium supplementation remain to be determined.

Previous studies by our group showed that SeMet is able to block cell-cycle progression at specific checkpoints, which might be explained by a decrease in cyclin B and Cdc2 kinase activity (25). In studies by other groups, SeMet at a concentration of 500 μM induced cell-cycle arrest and phosphorylation of Cdc2 at Tyr-15 in prostate tumor cells (42). At 150 μM SeMet was also shown to be able to inhibit the growth of LNCaP and decrease cyclin D1 and cyclin D3, which were associated with a decrease in cyclin B and Cdc2 kinase (25). In Xenopus egg extracts, G2 arrest by Wee 1 is associated with Wee 1 RNA levels in SeMet-treated cells during growth arrest. We speculate that if ERK activates Wee1 it is phosphorylated (50). We provide evidence that ERK activation is associated with phosphorylation of Cdc2 on Tyr-15 (42). The phosphorylation of Cdc2 on Tyr-15 has been implicated in the G2/M checkpoint (27,28). Wee1 kinase is known to phosphorylate Cdc2 at Tyr-15. We also observed an increase in Wee1 RNA levels in SeMet-treated cells during growth arrest. In Xenopus egg extracts, G2, arrest by Wee 1 is associated with p42 MAPK (50). We speculate that if ERK activates Wee1 it is through an indirect mechanism involving induction of Wee1 at the transcriptional level. Although Wee1 kinase RNA levels were found to be increased in our studies, whether it is ERK-related and leads to subsequent phosphorylation of Cdc2 requires further study. The possible role of the endoplasmic reticulum- and Golgi complex-bound protein kinase MYT1 as another candidate responsible for phosphorylation of Cdc2 (51,52) and the destruction of CDC25 phosphatases, which have been studied previously in response to mitotic DNA damage, or H3 phosphorylation (53), are also under current investigation in relation to SeMet.

Although ERK is thought to mediate mitogenic responses, there are several reports indicating that persistent ERK activation can lead to cell-cycle arrest (54,55). In the present study we provide evidence that ERK activation is associated with SeMet induced cell growth inhibition. A role for MAPK pathway signaling in growth arrest or cellular differentiation is not unprecedented. For example, a synthetic vitamin K analog compound 5 (Cpd 5) or 2-(2-mercaptoethanol)-3-methyl-1,4-naphthoquinone is an inhibitor of protein phosphatase Cdc25A and causes persistent activation of ERK and cell growth inhibition in Hep3B hepatoma cells (11). ERK activation also
appears to mediate cell-cycle arrest and apoptosis after DNA damage independent of p53 (10).

We observed phosphorylation of histone H3 at Ser-10 upon SeMet treatment and U0126 caused a partial reduction of histone H3 phosphorylation. Stimulation of the ERK pathway results in an increase in phosphorylation of histone H3 during the activation of immediate early genes expression. These genes are activated directly and require no new transcription or translation for their induction. Phosphorylation at Ser-10 on histone H3 is functionally linked to Gcn5-mediated acetylation at lysine 14. Mol. Cell, 5, 917–926.

In summary, we demonstrate that SeMet activation of ERK leads to histone H3 phosphorylation via p90RSK in HCT116 cells. We hypothesize that histone H3 phosphorylation leads to chromatin remodeling, which leads to changes in gene expression, and in turn ultimately results in growth inhibition. We also show for the first time a dose-dependent activation of this pathway with physiologic concentrations of SeMet seen in human clinical trials. However, the molecular mechanism leading to ERK activation by SeMet (at physiologic concentrations) will require further study.

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References

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