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Selenomethionine induces polyamine biosynthesis in regenerating rat liver tissue

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Summary. Our study was undertaken to elucidate the effects of selenomethionine (SeMet) on polyamine metabolism in regenerating rat liver tissue, as useful model of rapidly growing normal tissue. We have examined the levels of spermine, spermidine and putrescine in liver tissue. At the same time we have evaluated the activities of polyamine oxidase (PAO) and diamine oxidase (DAO), the catabolic enzymes of polyamine metabolism.

The obtained results suggest that polyamine levels in regenerating liver tissue, at 7th day after two-thirds partial hepatectomy, were higher in comparison with control group. The administration of selenomethionine to hepatectomized animals during seven days, in a single daily dose of 2.5 μg/100 g body weight, increases the amount of spermine and spermidine; the level of putrescine does not change under the influence of SeMet in regenerating rat liver tissue.

PAO activity is lower in regenerating hepatic tissue than in control group. Supplementation of hepatectomized animals with SeMet significantly decreases the activity of this enzyme. DAO activity was significantly higher in hepatectomized and in operated animals treated with SeMet compared to the sham-operated and control ones.

The differential sensitivity observed in our model of highly proliferating normal tissue to SeMet, compared with the reported anticancer activity of this molecule is discussed.

Keywords: Se-methionine – Polyamines – Polyamine oxidase – Diamine oxidase – Liver regeneration – Rats

Introduction

Polyamines, spermine, spermidine and putrescine are naturally-occurring cellular components in all living cells. These aliphatic cations are essential for life. A large body of data indicates that polyamines are important for cell proliferation (Raina and Janne, 1975; Tabor and Tabor, 1984; Pegg, 1986; Heby and Persson, 1990). Polyamine levels increase in rapidly growing tissues like regenerating rat liver, chick embryo, as well as in malignant tissues. Cancer cells have increased levels of polyamines compared to that of normal cells (Thomas and Thomas, 2001; Russell, 1973; Seiler et al., 1998). Selenium is an essential trace element found in cereals, wheat, dairy products, meat, and fish. This micronutrient may prevent carcinogenesis through several biochemical pathways; one suggested pathway is enhanced apoptosis (Kajander et al., 1991; Schrauzer, 2001). The efficacy of dietary selenium supplementation is currently being evaluated in intervention trials. SeMet was the predominant form of selenium in dietary supplementation (Schrauzer, 2000; Connelly-Frost et al., 2006).

The biological mechanisms underlying the cancer chemopreventive effects of selenium supplementation have yet to be elucidated. The cancer chemopreventive effect of selenium supplementation is well known. An anticarcinogenic effect of selenium compounds has been demonstrated in several animal model forms of cancer. The tumor preventive effect of selenium compounds has also been shown in human cancers and supplementation is likely to be officially recognized as a mean of lowering cancer risk.

SeMet is present in broccoli, garlic and onions (Finley et al., 2000). Selenium metabolism and polyamine biosynthesis are linked in their common requirement for S-adenosylmethionine (Redman et al., 1997). SeMet inhibited the growth of human tumor cell lines in micromolar range. Administration of SeMet to cancer cell results in apoptotic cell death and aberrant mitoses (Redman et al., 1997; El-Bayoumy and Sinha, 2004).

The aim of our study was to elucidate the influence of SeMet on polyamine intracellular levels and polyamine oxidase (PAO) and diamine oxidase (DAO) activities in regenerating rat liver tissue, as a good model of highly-proliferating tissue. The findings presented appear to demonstrate the differential sensitivity of tumor and normal cells to SeMet.

Materials and methods

Male albino Wister rats weighing 150–180 g were used for this study. Partial hepatectomy was performed by resection of two-thirds of liver tissue mass (67%), according to the method of Higgins and Anderson (1931), under Kethalar anesthesia. Immediately after operation, the hepatectomized animals were divided in two parts: the animals that received selenometionine (“Sigma”) intraperitoneally in a single daily dose of 2.5 μg/100 g body weight, and those that received 0.9% NaCl instead of the drug. The animals received the same dose of the drug for seven days. The last dose of selenomethionine was applicated on the 7th day after hepatectomy, 1 h before sacrificing. The control group and sham operated animals received 0.9% NaCl. Sham hepatectomy consisted of laparatomy with removal of a small piece of omental fat. Data from the sham hepatectomy experiments are not presented because there were no significant differences in any of the measured parameters compared to control. The animals were killed by decapitation. Livers were removed quickly, excess blood removed by blotting, and liver pieces frozen at −70 °C for up to one week, until they were analyzed for polyamine amount, diamine and polyamine oxidase activities and total protein content. The liver tissue was cut in small pieces and homogenized in ice-cold water. The amount of polyamines was investigated with butanol extraction followed by electrophoresis (Russell et al., 1970). For the estimation of DAO and PAO activity the liver tissue was cut in small pieces and homogenized in ice-cold water. The homogenates (10% w/v) were centrifuged at 1500 × *g* for 10 min at 4 °C. The resulting supernatants were used for the investigation of enzyme activities. DAO (putrescine-dihydrochloride was used as a substrate) and PAO (spermine-tetrahydrochloride was used as a substrate) activities were measured by spectrophotometric method of Bachrach and Reches (1966), modified by Quash et al. (1979). One unit of enzyme activity was defined as an increase in optical density of 0.100 at 660 nm. Proteins were determined according to the method of Lowry et al. (1951). The obtained results were statistically analyzed using Student’s T-test.

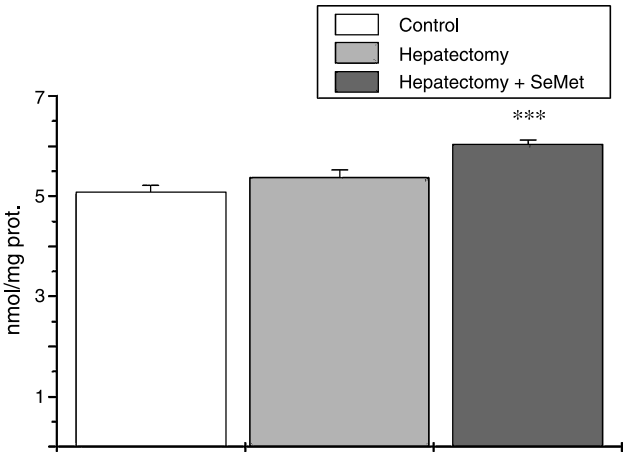

Fig. 1. SeMet effects on spermine levels in rat liver tissue. Male albino rats underwent under partial hepatectomy according to the method of Higgins and Anderson (1931). A group of them received SeMet intraperitoneally for seven days (2.5 μg/100 g body weight). Livers were removed quickly and frozen at −70 °C for up one week, until they were analyzed for polyamines (Russell et al., 1970). Data are expressed as nmoles/mg of total proteins and are the mean of four determinations ± SD

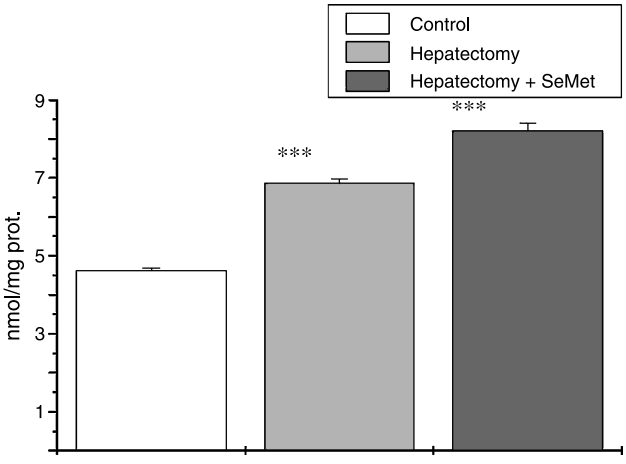

Fig. 2. SeMet effects on spermidine levels in rat liver tissue. Male albino rats underwent under partial hepatectomy according to the method of Higgins and Anderson (1931). A group of them received SeMet intraperitoneally for seven days (2.5 μg/100 g body weight). Livers were removed quickly and frozen at −70 °C for up one week, until they were analyzed for polyamines (Russell et al., 1970). Data are expressed as nmoles/mg of total proteins and are the mean of four determinations ± SD

Results

Effect of partial hepatectomy on liver polyamine pools

Partial hepatectomy does not change spermine and putrescine concentrations 7 days after the operation; at the same time of regenerating period, spermidine levels significantly increase in comparison with the control (sham operated) animals (Figs. 1–3).

Effect of SeMet on polyamine pools after partial hepatectomy

The supplementation of hepatectomized animals with SeMet during seven days of regenerating period increases spermine and spermidine levels significantly on 7th day after hepatectomy compared to those of hepatectomized animals that received 0.9% NaCl instead of the drug (Figs. 1 and 2). At the same time, the putrescine level observed in regenerating liver tissue on the 7th day after hepatectomy does not change under the influence of SeMet (Fig. 3).

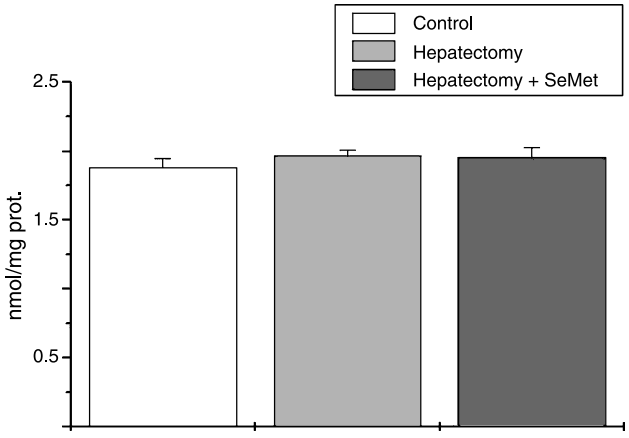

Fig. 3. SeMet effects on putrescine levels in rat liver tissue. Male albino rats underwent under partial hepatectomy according to the method of Higgins and Anderson (1931). A group of them received SeMet intraperitoneally for seven days (2.5 µg/100 g body weight). Livers were removed quickly and frozen at −70 °C for up one week, until they were analyzed for polyamines (Russell et al., 1970). Data are expressed as nmoles/mg of total proteins and are the mean of four determinations ± SD

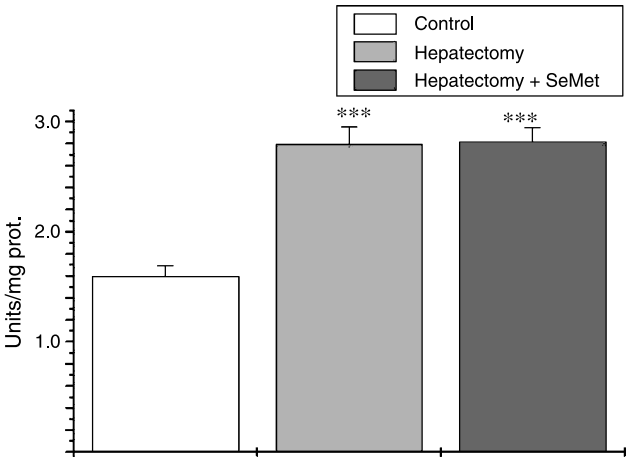

Fig. 5. SeMet effects on DAO activity in rat liver tissue. Male albino rats underwent under partial hepatectomy according to the method of Higgins and Anderson (1931). A group of them received SeMet intraperitoneally for seven days (2.5 µg/100 g body weight). Livers were removed quickly and frozen at −70 °C for up to 1 week, until they were analyzed for DAO activity by a modified spectrophotometric method (Quash et al., 1979). One unit of enzyme activity was defined as an increase in optical density of 0.100 at 660 nm. Data are expressed as units/mg of total proteins and are the mean of four determinations ± SD

Effects of partial hepatectomy and Se-methionine on liver polyamine oxidase and diamine oxidase activities

PAO activity decreases in regenerating rat liver tissue on the 7th day after two-third partial hepatectomy. The supplementation of hepatectomized animals with SeMet during seven days causes significant decrease of PAO activity in hepatectomized animals, compared to the control ones (Fig. 4). DAO activity was significantly higher in hepatectomized animals (Fig. 5) and in those operated animals treated with SeMet compared to the sham operated and control ones.

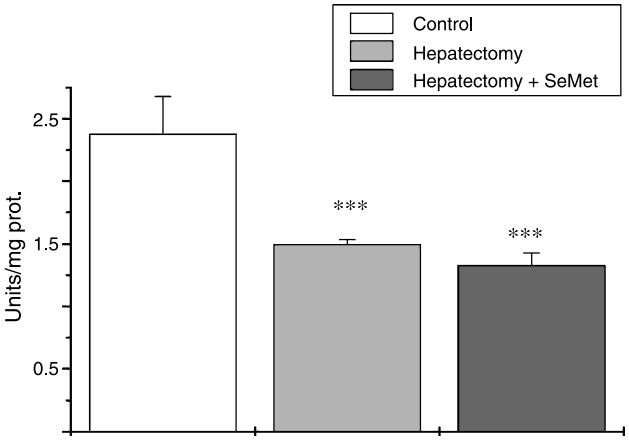

Fig. 4. SeMet effects on PAO activity in rat liver tissue. Male albino rats underwent under partial hepatectomy according to the method of Higgins and Anderson (1931). A group of them received SeMet intraperitoneally for seven days (2.5 µg/100 g body weight). Livers were removed quickly and frozen at −70 °C for up to 1 week, until they were analyzed for PAO activity by a modified spectrophotometric method (Bachrach and Reches, 1966; Quash et al., 1979). One unit of enzyme activity was defined as an increase in optical density of 0.100 at 660 nm. Data are expressed as units/mg of total proteins and are the mean of four determinations ± SD

Discussion

Polyamines, putrescine, spermidine, and spermine, constitute a group of cell components important in the regulation of cell proliferation and differentiation. There is also evidence suggesting a role for polyamines in programmed cell death. Polyamines appear to play an essential role in the metabolic processes involved in cell growth and division, including DNA, RNA, and protein synthesis, embryonic development and cell differentiation. Polyamine content is increased in many cancers (Heby, 1981; Wallace et al., 2003; Kalač and Krausova, 2005; Moinard et al., 2005; Eugene et al., 2004). For many years, both in epidemiology and in vitro studies, selenium supplementation has been shown to work as an anticarcinogenic agent. Selenium supplementation has recently been shown to decrease total cancer incidence. However, the mechanism of action of selenium as an anticarcinogenic agent has yet to be elucidated (Redman et al., 1998). Previous studies in animals and humans have shown that selenium compounds can prevent cancer development. The results suggest that selenium is able to reduce the risk for liver

cancer even when it is used only during a short period of time covering the promotion phase of the carcinogenic process. Chemically induced hepatocarcinogenesis may be prevented by selenium supplementation both during promotion and progression phase (Zeng, 2002; Bergman et al., 2005). Data support the activation of the tumor suppressor protein p53, increased resistance against oxidative stress due to induction of selenoproteins, as well as interactions with cell-cycle signal transduction (Bergman et al., 2005). Methionine adenosyltransferase (MAT) catalyzes the formation of S-adenosylmethionine (SAM), the principal biological methyl and propylamine donor, essential to polyamine synthesis and normal cell function (Tabor and Tabor, 1984). The examination of changes in hepatic MAT gene expression after two-thirds partial hepatectomy (PH) in rats showed that MAT mRNA levels increased from 3 to 6 h up to 4 days after PH (Huang et al., 1998). We used regenerating rat liver tissue as a good experimental model for rapidly growing tissue. The increase of polyamine concentration in liver tissue after two-thirds partial hepatectomy, observed in our experiment may be explained by higher activity of methionine adenosyltransferase (MAT). Methionine adenosyltransferase activity was not altered by SeMet treatment (Kajander et al., 1990). Redman et al. (1998) found that SeMet inhibited tumor growth (both in lung and colon cancer cells) in a dose-dependent manner. At least, part of anticarcinogenic effects of selenium supplementation might be due to depletion of polyamine levels (Redman et al., 1997). Since in cancer cells SeMet supplementation led to a decreased proliferation and to a depletion of polyamines (Redman et al., 1997), the differential sensitivity showed by our findings in a model of normal growth is a clear evidence of the impairment of the SeMet induction of MAT in cancer cells (Kajander et al., 1990).

According to these results and several others literature data, about antiproliferative effect of SeMet, our findings appear clearly controversial. Indeed we observe an increase of polyamine (spermidine and spermine) levels in regenerating liver tissue of rats treated with SeMet for seven days. This increase of spermine and spermidine may be explained considering that most of the ingested SeMet is either metabolized directly to reactive forms of selenium or stored in place of methionine in body proteins (Schrauzer, 2000). It is known that the methionine, as S-adenosylmethionine i.e. ''active methionine'' serves as propylamine donor during the synthesis of spermidine and spermine. The by-product of this process is 5′-methylthioadenosine. Our results suggest that SeMet could be used for polyamine synthesis in the same manner as ''active methionine''. SeMet is incorporated primarily into skeletal muscle, erythrocyte, pancreas, liver and kidney proteins. SeMet is effectively metabolized to S-adenosylmethionine analogue, and this molecule is further metabolized through transmethylation reactions and in polyamine synthesis, similarly to the corresponding sulfur metabolites of methionine. SeMet is activated initially by adenosylation, demethylated in analog to methionine without involving SeMet-specific enzymes (Schrauzer, 2000). L-SeMet is metabolized to a selenium analogue of S-adenosylmethionine approximately as effectively as the natural sulfur analogue of methionine (Schrauzer, 2000; Kajander et al., 1990). Evidence for degradation of SeMet to methylselenol (CH_3–SeH) by lyase action has also been obtained (Schrauzer, 2000). According to these data, supplementation of hepatectomized animals with SeMet brings higher amounts of substrate for spermidine and spermine biosynthesis. SeMet is activated in the reaction with ATP and converted to seleno-adenosyl methionine. After reaction of decarboxylation Se-adenosyl methionine serves as propylamine donor for synthesis of spermidine, and spermine, as well (Tabor and Tabor, 1984).

The principal catabolic pathway for polyamines involves oxidative deamination of spermine and spermidine by PAO (EC 1.5.3) to putrescine and 3-aminopropionaldehyde (Holtta, 1977; Seiler, 1995; Vujić et al., 2003). Putrescine is substrate for DAO (EC 1.4.3.6). The enzymes are widely distributed in animal tissues, especially liver tissue (Seiler and Raul, 2005). Our results show that SeMet supplementation to hepatectomized animals causes the decrease of PAO activity. On the contrary, DAO activity increases under the influence of SeMet in regenerating rat liver. We may explain these changes of these enzyme activities by SeMet metabolic events in animal tissues. The estimation of SeMet kinetics (Paterson et al., 1989) shows that ingested SeMet is either metabolized directly to reactive forms of selenium or stored at places of methionine in body proteins (Schrauzer, 2000). SeMet may replaces methionine in the vicinity of the active site, affecting the activity of the catabolic enzymes, Since CH_3–Se group of SeMet is more hydrophobic than the CH_3–S-moiety of methionine, substrate access may be inhibited, altering the kinetic parameters of enzymes (Schrauzer, 2000). These changes may be responsible for the decrease of PAO activity and for the increase of DAO activity. Taken together our data indicate that in rapidly-growing tissues, SeMet supplementation is an inducers of polyamine biosynthesis. Since SeMet belongs to the group of amino acids which cannot be synthesized in higher animals and humans, it may be also needed for some specific

functions in the organism. For example, it has been suggested to act as a cellular antioxidant. In the reaction with peroxynitrite, SeMet oxide is formed, which is then reduced back to SeMet by ascorbic acid (Schrauzer, 2000). Actually, beneficial effects of SeMet on the human body are under consideration.

Basic research could contribute in gaining new knowledge in our understanding of selenium biochemistry and its relation to polyamine metabolism in anticancer efficacy and regulation of cell growth (Ip, 1998). However, the differential sensitivity of normal growing cells compared with the cancer counterpart, toward SeMet, may be an usefull application for a more specific anticancer therapy.

References

Bachrach U, Reches B (1966) Enzymic assay for spermine and spermidine. Anal Biochem 17: 38–48

Bergman BL, Torndal1 UB, Eken SC, Nyström C, Capitanio A, Larsen HE, Björnstedt MLC, Eriksson CL (2005) Selenium prevents tumor development in a rat model for chemical carcinogenesis. Carcinogenesis 26: 125–131

Connelly-Frost A, Poole C, Jessie A, Satia AJ, Kupper LL, Robert C, Millikan CR, Sandler SR (2006) Selenium, apoptosis, and colorectal adenomas. Cancer Epidemiol Biomark Prevent 15: 486–493

El-Bayoumy K, Sinha R (2004) Mechanisms of mammary cancer chemoprevention by organoselenium compounds. Mutat Res 551: 181–197

Eugene W, Gerner WE, Meyskens LF (2004) Polyamines and cancer: old molecules, new understanding. Nature Rev Cancer 4: 781–792

Finley JW, Davis CD, Feng Y (2000) Selenium from high selenium broccoli protects rats from colon cancer. J Nutr 130: 2384–2389

Heby O (1981) Role of polyamines in the control of cell proliferation and differentiation. Differentiation 19: 1–20

Heby O, Persson L (1990) Molecular genetics of polyamine synthesis in eukaryotic cells. Trends Biol Sci 15: 152–158

Higgins GH, Anderson RM (1931) Experimental pathology of the liver. Restoration of the liver in the white rat following partial surgical removal. Arch Pathol 12: 186–202

Holtta E (1977) Oxidation of spermidine and spermine in rat liver: purification and properties of polyamine oxidase. Biochemistry 16: 91–100

Huang ZZ, Mao Z, Cai J, Lu CS (1998) Changes in methionine adenosyltransferase during liver regeneration in the rat. Am J Physiol Gastrointest Liver Physiol 275: G14–G21

Ip C (1998) Lessons from basic research in selenium and cancer prevention. J Nutr 128: 1845–1854

Kajander EO, Harvima RJ, Kauppinen L, Akerman KK, Martikainen H, Pajula RL, Karenlampi SO (1990) Effects of selenomethionine on cell growth and on S-adenosylmethionine metabolism in cultured malignant cells. Biochem J 267: 767–774

Kajander EO, Harvima RJ, Eloranta TO, Martikainen H, Kantola M, Karenlampi SO, Akerman K (1991) Metabolism, cellular actions and cytotoxicity of selenomethionine in cultured cells. Biol Trace Elem Res 28: 57–68

Kalač P, Krausova P (2005) A review of dietary polyamines: Formation, implications for growth and health and occurrence in foods. Food Chem 90: 219–230

Lowry O, Rosenbrough N, Farr A, Randall R (1951) Protein measurement with Folin phenol reagent. J Biol Chem 193: 265–275

Moinard CH, Cynober L, De Brandt JP (2005) Polyamine: metabolism and implications in human diseases. Clin Nutr 24: 184–197

Patterson HB, Levander AO, Helzlsouer K, McAdam AP, Lewis AS, Taylor RP, Veillon C, Zech AL (1989) Human selenite metabolism: a kinetic model. Am J Physiol Regul Integr Comp Physiol 257: R556–R567

Pegg EA (1986) Recent advances in the biochemistry of polyamines in eukaryotes. Biochem J 234: 249–262

Quash G, Keolouangkhot T, Gazzolo L, Ripoll H, Saez S (1979) Diamine oxidase and polyamine oxidase activities in normal and transformed cells. Biochem J 177: 275–282

Raina A, Janne J (1975) Physiology of the natural polyamines putrescine, spermidine and spermine. Med Biol 53: 121–147

Redman C, Xu MJ, Peng YM, Scott JA, Payne C, Clark LC, Nelson MA (1997) Involvement of polyamines in selenomethionine induced apoptosis and mitotic alterations in human tumor cells. Carcinogenesis 18: 1195–1202

Redman C, Scott JA, Baines AT, Basye JL, Clark LC, Calley C, Roe D, Payne CM, Nelson MA (1998) Inhibitory effect of selenomethionine on the growth of three selected human tumor cell lines. Cancer Lett 125: 103–110

Russell HD, Medina JV, Snyder HS (1970) The dynamics of synthesis and degradation of polyamines in normal and regenerating rat liver and brain. J Biol Chem 245: 6732–6738

Russell HD (1973) The roles of the polyamines, putrescine, spermidine and spermine in normal and malignant tissues. Life Sci 13: 1635–1647

Schrauzer NG (2000) Selenomethionine: a review of its nutritional significance, metabolism and toxicity. J Nutr 130: 1653–1656

Schrauzer NG (2001) Nutritional selenium supplements: product types, quality, and safety. J Am Coll Nutr 20: 1–4

Seiler N (1995) Polyamine oxidase, properties and functions. Prog Brain Res 106: 333–344

Seiler N, Atanassov C, Raul F (1998) Polyamine metabolism as target for cancer chemoprevention. Int J Oncol 13: 993–1006

Seiler N, Raul F (2005) Polyamines and apoptosis. J Cell Mol Med 9: 623–642

Tabor CW, Tabor H (1984) Polyamines. Ann Rev Biochem 53: 749–790

Thomas T, Thomas JT (2001) Polyamines in cell growth and cell death: molecular mechanisms and therapeutic applications. Cell Mol Life Sci 58: 244–258

Vujic S, Liang P, Diegelman P, Kramer LD, Porter WC (2003) Genomic identification and biochemical characterization of the mammalian polyamine oxidase involved in polyamine back-conversion. Biochem J 370: 19–28

Wallace MH, Frasaer VA, Hughes A (2003) A perspective of polyamine metabolism. Biochem J 376: 1–14

Zeng H (2002) Selenite and slenomethionine promote HL-60 cell cycle progression. J Nutr 132: 674–679

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