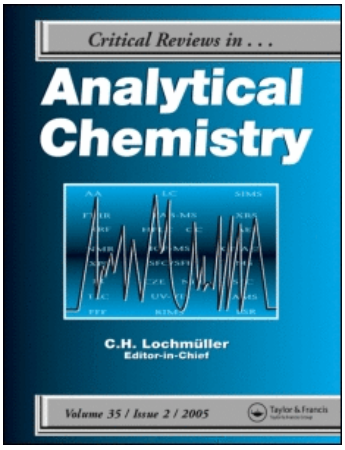

Critical Reviews in Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713400837

Current Status of Polyamine and Polyamine Analogs Analysis in Cancer Research

Armağan Önal[a]
[a] Faculty of Pharmacy, Department of Analytical Chemistry, Istanbul University, Istanbul, Turkey

Online publication date: 06 May 2010

To cite this Article Önal, Armağan(2010) 'Current Status of Polyamine and Polyamine Analogs Analysis in Cancer Research', Critical Reviews in Analytical Chemistry, 40: 2, 60 — 67
To link to this Article: DOI: 10.1080/10408340903018486
URL: http://dx.doi.org/10.1080/10408340903018486

Critical Reviews in Analytical Chemistry, 40:60–67, 2010

ISSN: 1040-8347 print / 1547-6510 online
DOI: 10.1080/10408340903018486

Current Status of Polyamine and Polyamine Analogs Analysis in Cancer Research

Armağan Önal
Istanbul University, Faculty of Pharmacy, Department of Analytical Chemistry, Istanbul, Turkey

The amino-acid-derived polyamines have been associated with cell growth and cancer and their concentrations in malignant tumors are extremely high compared to those in tissues that are histologically normal. Hence, polyamines have been considered as an interesting object for anticancer therapy. Analysis of polyamine levels in biological fluid can possibly provide helpful information on the types of cancer and progression phase of the disease. Numerous modern analytical methods, including high-performance liquid chromatography, gas chromatography, capillary electrophoresis, and other separation techniques have been widely utilized for analysing polyamine levels. In both tissue culture and experimental animal models, polyamine analogues restrain cell growth and destroy malignant cells. Determination of intracellular analogue contents is also crucial, since analogue accumulation is a causal factor for their antitumour effectiveness. In this review, the latest methods reported for polyamines and polyamine analogs in cancer research are discussed.

Keywords cancer, polyamines, polyamine analogs, analytical methods, biological fluids

INTRODUCTION

Cancer has been among our greatest health concerns for ages. It is an overall name given to a group of over 100 diseases where cell growth in a part of the body goes out of control (1). The reason for most cancer types are DNA damages that accrue throughout lifetime. DNA can be damaged by many things, including radiation like ultraviolet light and chemicals such as those in cigarette smoke. Despite the fact that the exact link between a bad diet and cancer risk still remains unknown, malnutrition also seems to be a possible reason of cancer development. Even some viruses are associated with cancer (2). Early diagnosis and rapid treatment are essential for fighting this disease in an effective manner. In spite of great advancements in the medical sciences, most tumors can still be revealed only in advanced or even final stages, which may be too late for treating them in a successful manner. In this respect, new, selective, and cheap tumor diagnostic techniques are the focuses of modern medicine.

Pre-cancer diagnosis has become a crucial subject in clinical and pre-clinical research, throughout the the advancement of analytical instrumentation and biochemistry. Studies on effective biomarkers for cancers are vital in pre-cancer screening or pre-cancer diagnosis since they give valuable information on the type of cancer, as well as on a patient's phase of advancement at very early stages. (3, 4).

Putrescine, spermidine, and spermine, which respectively contain two, three, or four charged amine groups, are the most common polyamines as biochemical markers of cancer. In cell proliferation, cell growth, and synthesis of proteins and nucleic acids, polyamines as cancer biomarkers take an important role (5–7). Among a number of biochemical changes in cancer cells, alterations in intracellular polyamine content is the most consistent since their concentrations in malignant tumors are notably higher than those in normal tissue. The increase depends on polyamine homeostasis loss which emerges during the dysregulation of cell proliferation (8–11). Polyamines have thus been seen as a crucial target for anti-cancer treatment (12, 13).

POLYAMINES METABOLIC PATHWAYS

Putrescine (PUT), spermidine (SPD), and spermine (SPM) polyamines and several polyamine metabolites including N-acetyl spermidine (N-acetyl SPD), N-acetyl spermine (N-acetyl SPM), and hydrogen peroxide (H2O2) naturally consist of elements that are essential for eukaryotic cell growth. The metabolism of polyamines are frequently deteriorated in cancer; therefore, polyamine function and metabolism is important targets for therapeutic intervention (14). There were numerous

Address correspondance to Dr. Armağan Önal, Istanbul University, Faculty of Pharmacy, Department of Analytical Chemistry, 34116, Beyazıt, Istanbul, Turkey; E-mail:armaganozkul@yahoo.com

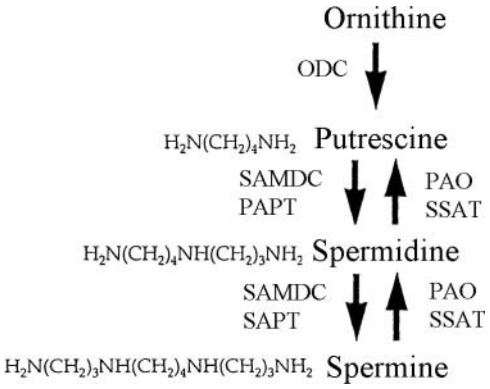

FIG. 1. Polyamine metabolism pathways. Enzymes involved are ornithine decarboxylase (ODC), *S*-adenosylmethionine decarboxylase (SAMDC), putrescine aminopropyl transferase (PAPT), polyamine oxidases (PAO), spermidine/spermine acetyl transferase (SSAT), S-adenosylmethionine decarboxylase (SAMDC), spermidine aminopropyl transferase (SAPT).

reviews reported on the polyamines metabolic pathway (15–25). Methabolic pathways of polyamines are briefly described in this document (Fig. 1).

Production of putrescine by the decarboxylation of the amino acid, ornithine by ornithine decarboxylase (ODC), constitutes the first critical step. Subsequent addition of an aminopropyl group to putrescine leads to the synthesis of spermidine and further addition of another aminopropyl group leads to the formation of spermine (26, 27). The aminopropyl group is derived by decarboxylation of *S*-adenosylmethionine (SAM), by (SAM) decarboxylase (DC) yields decarboxylated SAM, which gives its propyl amine moiety for spermidine and spermine formation respectively by spermidine synthase (SRM) and spermine synthase (SMS). Intracellular polyamine levels are also controlled by catabolism, permitting the conversion of spermine back to putrescine (27–29). In the retroconversion process, using acetyl-CoA to form *N*1-acetylspermidine and spermine, spermidine/spermine *N*1-acetyltransferase (SSAT) is the first step. The *N*1-acetyl derivatives are the preferred substrates of flavin adenine dinucleotide (FAD) -dependent polyamine oxidase (PAO), which respectively produce spermidine and putrescine (30). Figure 1, illustrates the pathways of polyamine metabolism.

POLYAMINE FUNCTIONS IN CANCER

Polyamines affect several carcinogeneous processes. Increased polyamine levels have been associated with increased cell proliferation, while decreases in apoptosis and increases in expression of genes affect tumor invasion and metastasis. Oppositely, a decrease in polyamine levels is associated with decreased cell growth, increased apoptosis, and decreased expression of genes affecting tumor invasion and metastasis (31, 32). This means that calculation of polyamine levels could be a utilized as a valuable tool in forecasting the effectiveness of the treatment.

A combination of SSAT, PAO, andpolyamine export leads to very low or non-detectable levelsof acetyl polyamines, in normal cells. Nevertheless, acetylpolyamines are detected in high concentrations in cancer cells and this provides an association between alterations in polyamine metabolism and carcinogenesis (33, 34).

Ornithine decarboxylase, which is a critical enzyme in the polyamine biosynthetic pathway, catalyzes ornithine's conversion into putrescine (35). ODC and polyamine levels are frequently elevated in tumor tissues relative to their normal counterparts (36, 37). DFMO was one of the first rationally designed anti-cancer drugs, and was directed against ODC (38). DFMO has been implemented in the establishment of the synthesis and the catabolism of the polyamine pathways, in the assessment of the polyamine-controlled mechanisms involving cell proliferation and differentiation, in the inhibition of cancer cell growth, in the improvement of existing human cancer therapies and in the development of new approaches in cancer chemotherapy, and even in cancer prevention programs. DFMO, however, has had dissatisfactory results in a majority of therapeutic attempts to use it as single drug, but based on its low toxicity, it may offer possible contributions in cancer chemoprevention. Numerous polyamine biosynthetic pathway drugs and polyamine analogues are actually being tested in human cancer patients. Up to now, the most successful in cell growth inhibition are polymine (PA) analogues bis(ethyl)-analogues of Spm and bis(ethyl)-analogues of Spd (39, 40). PA analogues regulate the enzymes of biosynthesis down, reduce the PA pools, and thus inhibit cell growth. These are subjected to individual and collective evaluations, and are assessed in combination with other anti-cancer drugs as well.

ANALYTICAL METHODS

Polyamines are interesting as biochemical markers of cancer, since some types of malignant cell proliferation are related to increases in cellular polyamine concentrations. Polyamine concentrations in human fluids normalize in patients after curative operations and these levels can further increase in patients who have proven tumor relapses and metastasis (41–45). In this context, it is important to determine these amines in human fluids, in order to screen for cancer screening, to assess effectiveness of therapy, and to identify relapses.

A number of studies have been reviewed for the detection of biogenic amines in biological fluids between 1975 and 2004 (46–48). In this manuscript, latest methods reported for polyamines and polyamine analogs in cancer research are reviewed.

N^1,N^{12}-diacetylspermine (DiAcSpm) and N^1,N^8-diacetylspermidine (DiAcSpd) are minor components of human urinary polyamine. Urinary polyamines analysis has demonstrated that the excretion of these diacetylpolyamines, in particular, into urine was frequently and markedly increased in association with every type of cancer so far examined (49).

Hiramatsu et al. (50) measured N^1,N^{12}-diacetylspermine (DiAcSpm) by ELISA in the urine of colon and breast cancer patients and compared the urinary DiAcSpm level with serum levels of other established tumor markers. Consequently, DiAcSpm was found to be a more sensitive marker compared to other established markers for colon and breast cancers and showed that it can proficiently detect cancers at early clinical phases. DiAcSpm was first detected and characterized by HPLC fractionation followed by enzymatic detection in the Kawakita et al. studies (51). More recently, however, antibodies highly specific for DiAcSpm were prepared, and an ELISA system applicable to determination of urinary DiAcSpm was established. DiAcSpm was elevated in hepatocellular carcinoma, nevertheless patients with liver cirrhosis also demonstrated considerably higher levels of DiAcSpm. Enjoji et al. had examined the clinical significance of urinary DiAcSpm using the ELISA system as a tumor marker for hepatocellular carcinoma (52).

Clinical implications of diacetylspermine, a urine tumor marker, were examined by comparing two conventional tumor markers, serum carcinoembryonic antigen and carbohydrate antigen 19-9, in 125 patients with pancreatobiliary diseases (53). The ELISA system was used for measuring diacetylspermine. Consequently, urine diacetylspermine is found to be a marker for pancreatobiliary carcinoma, which is as sensitive and specific as serum carbohydrate antigen 19-9.

Using ^{15}N-labded Ac_2Spm as the internal standard, an ion-spray ionization mass-spectrometric method for the identification of N^1,N^{12}-diacetylspermine (Ac_2Spm) was developed (54). Ac_2Spm concentrations in the urine obtained from 17 cancer patients measured by this technique directly correlated with the ones measured by means of ELISA, demonstrating the effectiveness of these two methods.

Stejskal et al. were investigaing diagnostic efficacy of diacetylspermine concentrations in the urine of individuals with urinary bladder cancer (55). Urine samples were used from 36 patients with urothelial tumors of the urinary bladder and from 30 patients with benign urological diseases. ELISA were performed for diacetylspermine from urine. As a result, urine *N*1,*N*12-diacetylspermine was probably not a useful marker for urinary bladder cancer.

Takahashi et al. analyzed the significance of the measurement of urine di-acetyl spermine as a cancer marker for colorectal cancer treatment (56). Urine and serum diacetyl spermine obtained from pre-operative and post-operative colorectal cancer patients were measured by ELISA. Their data showed that urine DiAcSpm predicted the prognosis after colorectal cancer surgery more exactly than serum CEA.

The cloning and initial characterization of human PAO were reported by Wang et al. (57).In homogenates, PAO activity was examined by a fluorometric method that calculates the H_2O_2 generated due to oxidation of spermine by turning homovanillic acid into a very fluorescent compound in the presence of horseradish peroxidase.

Effects of polyamine depletion by *a*-difluoromethylornithine on in vitro and in vivo biological properties of the 4T1 murine mammary cancer cells were investigated by Jun et al. (58) Polyamine depletion by DFMO has been shown to decrease pulmonary and bone metastasis from human breast cancer cell xenografts. HPLC was utilized for determining polyamine levels. This research demonstrated that inhibition of polyamine biosynthesis with DFMO noticeably suppressed in vitro proliferation and invasiveness of the 4T1 murine mammary cancer cell.

The inhibitory effect of ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase bi-antisense AdoMetDCas (Ad-ODC-AdoMetDCas) virus on lung cancer cell A-549 were investigated by Qi-Feng et al. (59). An HPLC method was used to determine the expression of the polyamine content in A-549 cells. Ad-ODC-AdoMetDCas has a significant inhibitory effect on proliferation and invasion of lung cancer cells and has therapeutic potential for the treatment of lung cancer.

Mateva et al. reported a HPLC method with fluorescent detection for simultaneous analysis of plasma and urine free catecholamines, metanephrines, and polyamines without prior treatment procedures via precolumn derivatization with Fmoc-Cl. The proposed method for simultaneous analysis of CA, PA, and MN is especially appropriate for early diagnostics of diseases, where changes in concentrations of these substances are expected to occur (60).

A single hollow fiber supported liquid membrane (SLM) extraction technique of polyamines, which is followed by simple pre-column derivatization with tosyl chloride and HPLC-UV analysis, was explained by Dziarkowska et al (61). In order to develop an SLM extraction method of polyamines from urine and plasma, the transport of polyamines through supported liquid membrane immobilized in a single hollowfiber were studied in this study.

SAM486A is an inhibitor of the polyamine biosynthetic enzyme *S*-adenosylmethionine decarboxylase (SAMDC). Siu et al. (62) performed studies to characterize the toxicity profile and the pharmacological behavior and to determine the maximum tolerated dose of SAM486A administered by a 1-h i.v. infusion daily for 5 days every 3 weeks in patients with advanced cancer. SAM486A was detected using a HPLC method with a UV detector monitoring at a wavelength of 230 nm.

Polyamines' utility as biomarkers of the evolution of the murine L5178Y lymphoma is reported in different body fluids, cells, and tissues (63). Findings were also applied to anti-tumor effect of Bursera fagaroides. Cation exchange HPLC was used to determine the PA levels in urine, peritoneal cells, circulating lymphocytes, spleenocytes, mesothelium, and liver of BALB/c mice at days 10, 17, and 24 of tumoral evolution. PA levels were also measured in urine from mice treated, intraperitoneally or orally, with the hydroalcoholic extract of the bark of B. fagaroides. Spd and Spm urinary levels were not detectable, while Pu increase in urine is the best biomarker to detect lymphoma growth. Furthermore, Pu urinary levels decreased significantly in mice treated intraperitoneally with B. fagaroides.

Five cyclopropane-containing polyamine analogues, 7, 10, 18, 27, and 32, have been evaluated for their anti-neoplastic activities against DU-145 human prostate cancer tumors implanted in nude mice (64). A HPLC fluorescence monitor was used for polyamine analysis.

Arimural et al. (65) investigated involvement of polyamines in evening primrose extract (EPE)-induced apoptosis in Ehrlich ascites tumor cells. To determine the effect of EPE on intracellular polyamines concentration, their levels were assayed by HPLC using fluorescence dedector. The fluorescence was measured at excitation and emission wavelength of 365 and 455 nm, respectively. The results showed that EPE caused a significant decrease in putrescine, spermidine, and spermine levels within 30 minutes after the addition.

Deng et al. developed a HPLC method for the determination of biogenic amines in human plasma of three healthy volunteers and four cancer patients, based on the precolumn derivatization with N-hydroxysuccinimidyl fluorescein-O-acetate (66). Weiss et al. examined the correlation of ODC activity and polyamine levels with tumor stage and grade in terms of sample recruitment (67). In tissue samples from 64 patients with colorectal adenocarcinomas, HPLC was utilized for determinig polyamines levels. It is detected that polyamine content is correlated with the tumor stage.

In the Inoue et al. study (68), a HPLC technique for the simultaneous determination of free and N-acetylated polyamines in urine with fluorescence detection after pre-column derivatization with 4- (5,6-dimethoxy-2-phthalimidinyl)-2-methoxyphenylsulfonyl chloride was described. The fluorescent derivatives were separated on a reversed-phase column with a gradient elution utilizing water–acetonitrile–methanol at 50°C and detected by fluorescence measurements at 318 nm (excitation) and 406 nm (emission). The detection limits of the polyamines and N-acetylated polyamines were 0.7–4.5 fmol/injection.

HPLC assay with fluorescence detection was developed for the determination of the polyamines putrescine, spermidine, spermine in samples of human spinal cord, cerebellum, cerebrospinal Xuid, skeletal muscle, and muscle microdialysates without an extensive sample preparation (69). The pre-column derivatization was performed with 9-fluorenylmethyl chloroformate. All polyamines were separated within 35 minutes. The author stated that this assay is a useful tool in the ongoing search for functions of polyamines in cancer research.

A HPLC method for simultaneous determination of amino acids, polyamines putrescine, spermidine, spermine, catecholamines, and metanephrines, in human body fluids was reported (70). Acidic (pH = 2.1) and basic (pH = 8.4) ammonium acetate buffers that contain varying amounts of dibutylamine were used as eluents. Perchloric acid, which also acts as an ion-pairing agent, was utilized for achieving a gradual increase of pH of mobile phase.

Liquid chromatography-mass spectrophotometry (LC-MS) analysis was developed to perform extractive carbamoylation of polyamines for the investigation of their biological roles in both urine and serum samples obtained from breast cancer patients (71). This technique was utilized for monitoring the polyamine concentration range in urine and serum samples obtained in 30 breast cancer patients and 30 age- and gender-matched normal controls. No significant difference between the urine of breast cancer patients and the controls in urinary polyamine levels was detected. Comparably, the concentrations of 1,3-Diaminopropane, Put, Spm, and $N-$acetylspermidine levels in serum were significantly increased in breast cancer patients.

A time-of-flight mass spectrometer with electrospray ionization interface method to determine the concentrations of spermidine and spermine in mammalian cells was described by Samejima et al. (72). Data acquisition of one sample required approximately 2 minutes. This method was successfully applied for the identification of lowered spermidine and spermine contents in cultured cells under the inhibition of aminopropyl transferases.

A reversed phase liquid chromatography–electrospray ionization-tandem mass spectrometric method (RP-LC–ESI-MS/MS) was developed to separate and detect polyamines (73). Prior to MS/MS analysis, a complete chromatographic separation of polyamines was achieved by a linear gradient elution using heptafluorobutyric acid as a volatile ion-pair modifier, and signal suppression was prevented by post-column addition of 75% propionic acid in isopropanol to the column flow.

Byun et al. (74) were was designed to evaluate serum polyamine levels along with operative conditions in both pre- and post-menopausal BCa patients compared to healthy subjects using a validated liquid chromatography–tandem mass spectrometry (LC–MS/MS). Serum polyamines were higher in pre-menopausal BCa patients, while those in post-menopausal patients were similar to the controls. Also, all polyamine levels decreased slightly in post-surgery patients and they were comparable to healthy subjects. The result suggests that serum polyamine levels correlate with estrogen levels, along with menopausal status, due to the association of estrogen-induced cell growth and ODC activity.

Simultaneous determination of MeCy5-OSu-derivatized polyamines spermine, spermidine, cadaverine, and putrescine based on the separation by capillary electrophoresis combined with diode LIF detection has been accomplished by Fu et al. (75). The analysis of polyamines in erythrocytes can be used for studying the relationship between their changes and the carcinogenesis process involved in erythrocytes.

Liu et al. (76) reported separation and detection of catecholamines and polyamines in PC-12 cell extracts by using micellar electrokinetic capillary chromatography (MECC) with UV absorption detection. The author stated that this technique can be easily applied to polyamine-related anti-cancer drug studies or clinical follow-ups after each dosage of these anticancer drugs, since these drugs not only have great inhibition on polyamine levels in blood, but also have a large influence on catecholamine levels in blood.

In the other report, electron-capture gas chromatographic analyses of putrescine, cadaverine, and spermidine in rat hepatic tissue was conducted after a 14-day administration of diamine oxidase (DAO) inhibitor aminoguanidine (77). Polyamine levels were measured using gas chromatography-electron capture detection. Significant increases in putrescine concentration were detected, but no such result was obtained in spermidine. In hepatic homogenates from aminoguanidine-treated rats, increased concentrations of cadaverine was also present.

As co-factors in the growth of cervical cancer, the potential role of estrogen, androgen, and polyamine metabolism were investigated by Lee et al. (78) Urine samples were obtained from patients with benign cervical disease and cervical cancer and from age-matched normal female subjects. An improved and sensitive gas-chromatographic with nitrogen/phosphorus-detection (GC/NPD) procedure was utilized for polyamine determination. The significant role that high activity of PAO in polyamine metabolism might play in the development of cervical cancer was detected.

The gas chromatography–mass spectrometry (GC–MS) in selected ion-monitoring (SIM) method for simultaneous determination of putrescine, spermidine, and spermine as N-heptafluorobutyryl derivatives in hair samples was developed by Li et al. They found that measuring hair polyamines may assist in early diagnosis and prevention of cancer in patients (79).

Paik et al. (80) investigated altered urinary PA levels from three different cancer cases at different intervals during the long-term week-day acupuncture treatments. Nine urinary PA levels from 16 normal and three cancer patients with different types of cancer were measured by GC-MS in SIM mode as N-ethoxycarbonyl-N-pentafluoropropionyl derivatives. Their levels measured at three follow-up stages for each patient were then normalized to the corresponding normal group means and plotted into star symbol patterns. The results showed that urinary PA profiling analysis combined with star graphic analysis appeared to be useful for monitoring metabolic changes induced by long-term acupuncture treatments in cancer.

The metabolomic approaches for mining biomarkers of women's cancers based on GC-MS and liquid chromatography-mass spectrometry combined with partial least squares-discriminant analysis are described by Woo et al. (81). A non-targeted metabolomic approach indicated that it is not only useful for diagnostic tools and patient stratification, but may be mapped on a metabolic network to reflect disease states.

CONCLUSION

Cancer has been among our greatest health concerns for ages. With the improvements in biochemistry and analytical instrumentation, pre-cancer diagnosis has become a hot point of clinical and pre-clinical research. Polyamines as cancer biomarkers play an important role in cell proliferation, cell growth, and synthesis of proteins and nucleic acids and have been seen considered as an interesting point for anti-cancer treatment. For screening polyamines in serum, urine, and tissues, many different techniques have been developed. Generally, they are analyzed through immunoenzymatic techniques, which are popular in bioanalytical laboratories. Polyamines do not absorb UV–vis light since they are very polar compounds; polyamines analysis in biological samples using classical analytical techniques such as chromatography or capillary electrophoresis need a lot of operation steps, which make them labor-and time-consuming. In this context, for polyamines analysis it is necessary to develop very simple, rapid, and cheap sample preparation techniques. Because polyamines are found in extremely low concentrations in biomatrix fluids and especially in urine, increasing the sensitivity of detection is an important matter. Therefore, hyphenated methods have been considered as a potential way to increase sensitivity. Consequently, the need for seeking new alternatives for polyamine analysis in cancer research is obvious.

REFERENCES

1. http://www.cancer.gov/cancertopics/what_is_cancer. April 2, 2010.
2. http://info.cancerresearchuk.org/cancerandresearch/learnaboutcancer/whatcausescancer/.
3. F. R. Hirsch, D. T. Merrick, and W. A. Franklin, Role of biomarkers for early detection of lung cancer and chemoprevention. *Eur. Resp. J.* 19 (2002):1151–1158.
4. P. R. Srinivas, B. S. Kramer, and S. Srivastava, Trends in biomarker research for cancer detection. *Lancet Oncol.* 2 (2001): 698–704.
5. A. E. Pegg, Recent advances in the biochemistry of polyamines in eukaryotes**.** *Biochem. J.,* 234 (1986):249–262.
6. C. W. Tabor and H. Tabor, Polyamines. *Annu. Rev. Biochem.* 53 (1984):749–790.
7. A. E. Pegg, Polyamine metabolism and its importance in neoplastic growth and a target for chemotherapy. *Cancer Res.* 48 (1988):759–774.
8. J. Jänne, H. Pös, and A. Raina, Polyamines in rapid growth and cancer. *Biochim. Biophys. Acta* 473 (1978):241–293.
9. T. Thomas and T. J. Thomas, Polyamine metabolism and cancer. *J. Cell. Mol. Med*. 7 (2003):113–126.
10. E. W. Gerner, and F. L. Meyskens, Polyamines and cancer: old molecules, new understanding. *Natl. Rev. Cancer* 4 (2004):781–792.
11. N. Seiler and F. Raul, Polyamines and apoptosis. *J Cell Mol Med*., 9 (2005): 623–642.
12. L. J. Marton and A. E. Pegg, Polyamines as targets for therapeutic intervention. *Annu. Rev. Pharmacol. Toxicol*. 35 (1995):55–91.
13. A. E. Pegg and P. P McCann, Polyamine metabolism and function: A brief review. *Am. J. Physiol*. 243 (1982):212–221.
14. R. A. Casero and L. J. Marton, Targeting polyamine metabolism and function in cancer and other hyperproliferative diseases. *Nature Reviews Drug Discovery* 6 (2007):373–390.
15. N. Seiler, Polyamine metabolism. *Digestion* 46 (2) (1990): 319–330.
16. F. R. Saunders and H. M. Wallace, Polyamine metabolism and cancer prevention. *Biochemical Society Transactions* 35 (2) (2007):364–368.

17. Y. Huanga, A. Pledgiea, R. A. Casero, Jr., and N. E. Davidson, Molecular mechanisms of polyamine analogs in cancer cells. *Anti-Cancer Drugs* 16 (2005):229–241.
18. W. E. Criss, A review of polyamines and cancer. *Turk. J. Med. Sci*. 33 (2003):195–205.
19. N. Palavan-Unsal, S. M. Aloglu-Senturk, and D. Arı san, The function of poliamine metabolism in pr ostate cancer, *Exp. Oncol.* 28 (3) (2006):178–186.
20. A. Gugliucci, Polyamines as clinical laboratory tools. *Clinica Chimica Acta* 344 (2004):23–35.
21. M. Linsalata and F. Russo, Nutritional factors and polyamine metabolism in colorectal cancer. *Nutrition* 24 (2008):382–389.
22. H. M. Wallace A. V. Fraser, and A. Hughes, A perspective of polyamine metabolism. *Biochem. J.* 376 (2003):1–14.
23. N. Seiler, How important is the oxidative degradation of spermine?: Minireview article. *Amino Acids* 26 (2004):317–319.
24. K. Igarashi and K. Kashiwagri, Polyamines: Mysterious modulators of cellular functions. *Biochem. Biophys. Res. Commun.* 271 (2000):559–564.
25. C. Moinard, L. Cynober, and J. P. de Bandt, Polyamines: Metabolism and implications in human diseases. *Clin. Nutr.* 24 (2005):184 –197.
26. D. H. Russell, Ornithine decarboxylase as a biological and pharmacological tool. *Pharmacology* 20 (1980):117–129.
27. T. Thomas and T. J. Thomas, Polyamines in cell growth and cell death: Molecular mechanisms and therapeutic applications. *Cell. Mol. Life Sci*. 58 (2001):244–258.
28. D. M. L. Morgan, Polyamines, an overview. *Mol. Biotechnol.* 11(1999):229–250.
29. R. A. Casero and A. E. Pegg, The turning point in polyamine metabolism. *FASEB J.* 7 (1993):653–661.
30. F. N. Bolkenius and N. Seiler, Acetyl derivatives as intermediates in polyamine catabolism. *Int. J. Biochem.* 13 (1981):287–292
31. N. A. Ignatenko, H. Zhang, G. S. Watts, B. A. Skovan, D. E. Stringer, and E. W. Gerner, The chemopreventive agent α-difluoromethylornithine blocks K ras dependent tumor formation and specific gene expression in Caco-2 cells. *Mol. Carcinog.* 39 (2004):221–233.
32. N. Babbar, N. A. Ignatenko, R. A. Casero, Jr., and E. W. Gerner, Cyclooxygenase-independent induction of apoptosis by sulindac sulfone is mediated by polyamines in colon cancer. *J. Biol. Chem.* 278 (2003):47762–47775.
33. A. N. Kingsnorth and H. M. Wallace, Elevation of monoacetylated polyamines in human breast cancers. *Eur. J. Cancer Clin. Oncol.* 21 (1985):1057–1062.
34. H. M. Wallace, J. Duthie, D. M. Evans, S. Lamond, K. M. Nicoll, and S. D. Heys, Alterations in polyamine catabolic enzymes in human breast cancer tissue. *Clin. Cancer Res.* 6 (2000):3657–3661.
35. P. H. M. Hoet and B. Nemery, Polyamines in the lung: polyamine uptake and polyamine-linked pathological or toxicological conditions. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 278 (2000):L417–L433.
36. G. M. LaMuraglia, F. Lacaine, and R. A. Malt, High ornithine decarboxylase activity and polyamine levels in human colorectal neoplasia. *Ann. Surg.* 204 (1986):89–93.
37. C. W. Porter, L. Herrera-Ornelas, P. Pera, N. F. Petrelli, and A. Mittelman, Polyamine biosynthetic activity in normal and neoplastic human colorectal tissues. *Cancer* 60 (1987):1275–1281.
38. B. W. Metcalf, P. Bey, C. Danzin, M. J. Jung, P. Casara, and J. P. Vevert, Catalytic irreversible inhibition of mammalian ornithine decarboxylase (E.C. + .1.1.17) by substrate and product analogs. *J. Am. Chem. Soc.* 100 (1978):2551–2553.
39. R. A. Casero, Jr., S. J. Ervin, P. Celano, S. B. Baylin, and R. J. Bergeron, Differential response to treatment with the bis(ethyl)polyamine analogues between human small cell lung carcinoma and undifferentiated large cell lung carcinoma in culture. *Cancer Res.* 49 (1989):639–643.
40. H. S. Basu, L. J. Marton, M. Pellarin, D. F. Deen, J. S. McManis, C. Z. Liu, R. J. Bergeron, and D. G. Feurstein, Design and testing of novel cytotoxic polyamine analogues. *Cancer Res.* 54 (1994):6210–6214.
41. N. E. Davidson, H. A. Hahm, D. E. McCloskey, P. M. Woster, and R. A. Casero, Jr., Clinical aspects of cell death in breast cancer:the polyamine pathway as a new target for treatment. *Endocr. Relat. Cancer* 6(1999):69–73.
42. N. Uehara, S. Shirakawa, H. Uchino, and Y. Saeki, Elevated contents of spermidine and spermine in the erythrocytes of cancer patients. *Cancer* 45 (1980):108–111.
43. D. H. Russell, Clinical relevance of polyamines as biochemical markers of tumor kinetics. *Clin. Chem.* 23(1) (1977): 22–27.
44. D. H. Russell, Clinical relevance of polyamines. *Crit. Rev. Clin. Lab. Sci.* 18 (1983):261–311.
45. A. E. Pegg and D. J. Feith, Polyamines and neoplastic growth. *Biochem. Soc. Trans*. 35 (2007):295–299.
46. M. Y. Khuhawar and G. A. Qureshi, Polyamines as cancer markers: Applicable separation methods. *J. Chromatogr. B* 764 (2001):385–407.
47. D. Teti, M. Visalli, and H. McNair, Analysis of polyamines as markers of (patho)physiological conditions. *Journal of Chromatography B* 781 (2002):107–149.
48. Y. Ma, G. Liu, M. Du, and I. Stayton, Recent developments in the determination of urinary cancer biomarkers by capillary electrophoresis. *Electrophoresis* 25 (2004):1473–1484.
49. M. Kawakita and K. Hiramatsu, Diacetylated derivatives of spermine and spermidine as novel promising tumor markers. *Journal of Biochemistry* 139 (3) (2006):315–322.
50. K. Hiramatsu, K. Takahashi, T. Yamaguchi, H. Matsumoto, H. Miyamoto. S. Tanaka, C. Tanaka, Y. Tamamori, M. Imajo, M. Kawaguchi, M.Toi, T. Mori, and M. Kawakita, N1,N12-diacetylspermine as a sensitive and specific novel marker for late-stage colorectal and breast cancers. *Clin. Cancer Res.* 11(8) (2005):2986–2990.
51. M. Kawakita, K. Hiramatsu, K. Takahashi, R. Yamada, M. Kawaguchi, N. Shinoura, T. Tanaka, K. Kariyone, Y. Tamamori, Y. Sasaki, and T. Mori, Urinary diacetylspermine: Its analysis and performance as a novel tumor marker. *Rinsho byori. The Japanese Journal of Clinical Pathology* 53(2) (2005):123–129.
52. M. Enjoji, M. Nakamuta, E. Arimura, S. Morizono, M. Kuniyoshi, M. Fukushima, K. Kotoh, and H. Nawata, Clinical significance of urinary N^1,N^{12}-diacetylspermine levels in patients with hepatocellular carcinoma. *International Journal of Biological Markers* 19(4) (2004):322–327.
53. K. Yamaguchi, M. Nakamura, K. Shirahane, H. Konomi, N. Torata, N. Hamasaki, M. Kawakita, and M. Tanaka, Urine diacetylspermine as a novel tumour maker for pancreatobiliary carcinomas. *Digestive and Liver Disease* 37 (2005):190–194.

54. M. Kobayashi, K. Samejima, K. Hiramatsu, and M. Kawakita, Mass spectrometric separation and determination of N^1,N^{12}-diacetylspermine in the urine of cancer patients. *Biol. Pharm. Bull.* 25 (3) (2002):372–374.
55. D. Stejskal, V. Humenansk, Z. Hanulova, R. Fiala, R. Vrtal, P. Solichova, and M. Karpisek, Evaluation of urine n1,n12-diacetylspermine as potential tumor marker for urinary bladder cancer. *Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech Repub.* 150(2) (2006):235–237.
56. K. Takahashi, K. Hiramatsu, M. Kawakita, T. Mori, T Yamaguchi, H. Matsumoto, H Miyamoto, and Y. Tamamori, The significance of urine di-acetyl spermine level as a cancer marker for colorectal cancer. *Rinsho Byori.* 52(4) (2004):332–335.
57. Y. Wang, W. Devereux, P. M. Woster, T. M. Stewart, A. Hacker, and R. A. Casero Jr. Cloning and characterization of a human polyamine oxidase that is Inducible by polyamine analogue exposure. *Cancer Research* 61 (2001):5370–5373.
58. J. Y. Jun, J. W. Griffith, R. Bruggeman, S. Washington, L. M. Demers, M. F. Verderame, and A. Manni, Effects of polyamine depletion by *a*-difluoromethylornithine on in vitro and in vivo biological properties of 4T1 murine mammary cancer cells. *Breast Cancer Res. Treat.* 107 (2008):33–40.
59. S. Qi-Feng, T. Hui, L. XianXi, Z. Bing; Z. Yan, and S. DongFeng, Inhibitory effect of ornithine decarboxylase and S-adenosylmethionine decarboxylase bi-antisense virus on lung cancer cell A-549. *Journal of Shandong University (Health Sciences)*. 45(8) (2007): 757–761.
60. M. Lyudmila, Sv. P. Stefan, St. L. Valentin, E. Atanaska, Z. Sabina, and Iv. M. Vanio, Simultaneous determination of free polyamines, catecholamines and metanephrines in plasma and urine. *Journal of Liquid Chromatography & Related Technologies* 31:(2008):2128–2140.
61. K. Dziarkowska, J. A. Jonsson, and P. P. Wieczoreka, Single hollow fiber SLM extraction of polyamines followed by tosyl chloride derivatization and HPLC determination. *Analytica Chimica Acta* 606(2008):184–193.
62. L. L. Siu, E. K. Rowinsky, L. A. Hammond, G. R. Weiss, M. Hidalgo, G. M. Clark, J. Moczygemba, L. Choi, R. Linnartz, N. C. Barbet, I. T. Sklenar, R. Capdeville, G. Gan, C. W. Porter, D. D. Von Hoff, and S. G. Eckhardt, A phase I and pharmacokinetic study of SAM486A, a novel polyamine biosynthesis inhibitor, administered on a dailytimes- five every-three-week schedule in patients with advanced solid malignancies. *Clinical Cancer Research*. 8, (2002):2157–2166.
63. R. Reynoso-Orozco, A. Santerre, J. I. Delgado-Saucedo, J. C. Solís, S. Velázquez-Magaña, and A. M. Puebla-Pérez, Polyamines as biomarkers of the antitumoral activity of Bursera fagaroides. *Interciencia* 33 (5) (2008):384–388.
64. B. Frydman, A. V. Blokhin, S. Brummel, G. Wilding, Y. Maxuitenko, A. Sarkar, S. Bhattacharya, D. Church, V. K. Reddy, J. A. Kink, L. J. Marton, A. Valasinas, and H. S. Basu, Cyclopropane-containing polyamine analogues are efficient growth inhibitors of a human prostate tumor xenograft in nude mice. *J. Med. Chem* 46 (2003):4586–4600.
65. T. Arimura1, A. Kojima-Yuasa1, Y. Tatsumi1, D. O. Kennedy, and I. Matsui-Yuasa1, Involvement of polyamines in evening primrose extract-induced apoptosis in Ehrlich ascites tumor cells. *Amino Acids* 28 (2005):21–27.
66. Y.-H. Deng, H.-S. Zhang, X.-L. Du, and H. Wang, Quantification of biogenic amines in human plasma based on the derivatization with N-hydroxy-succinimidyl fluorescein-O-acetate by high-performance liquid chromatography. *Journal of Separation Science* 31 (6–7) (2008):990–998.
67. T. S. Weiss, G. B. A. Buschauer, W. E. Thasler, D. Dolgner, H. Zirngibl, and K-W. Jauch, Polyamine levels of human colorectal adenocarcinomas are correlated with tumor stage and grade. *Int. J. Colorectal Dis.* 17 (2002):381–387.
68. H. Inoue, K. Fukunaga, S. Munemura, and Y. Tsuruta, Simultaneous determination of free and N-acetylated polyamines in urine by semimicro high-performance liquid chromatography using 4-(5,6-dimethoxy-2-phthalimidinyl)-2 methoxyphenylsulfonyl chloride as a fluorescent labeling reagent. *Analytical Biochemistry* 339 (2005):191–197.
69. T. Ekegren and C. Gomes-Trolin, Determination of polyamines in human tissues by precolumn derivatization with 9-Xuorenylmethyl chloroformate and high-performance liquid chromatography. *Analytical Biochemistry* 338 (2005):179–185.
70. V. Lozanov, B. Benkova, L. Mateva, S. Petrov, E. Popov, C. Slavov, and V. Mitev, Liquid chromatography method for simultaneous analysis of amino acids and biogenic amines in biological fluids with simultaneous gradient of pH and acetonitrile. *Journal of Chromatography B* 860 (2007):92–97.
71. J. A. Byun, S. H. Lee, B. H. Jung, M. H. Choi, M. H. Moon, and B. C. Chung, Analysis of polyamines as carbamoyl derivatives in urine and serum by liquid chromatography–tandem mass spectrometry. *Biomed. Chromatogr.* 22 (2008):73–80.
72. K. Samejima, M. Otani, Y. Murakami, T. Oka, M. Kasai, H. Tsumoto, and K. Kohda, Electrospray ionization and time-of-flight mass spectrometric method for simultaneous determination of spermidine and spermine. *Biol. Pharm. Bull.* 30(10) (2007):1943—1946.
73. M. R. Hakkinen, T. A. Keinanen, J. Vepsalainen, A. R. Khomutov, Lç. Alhonen, J. Janne, and S. Auriola, Analysis of underivatized polyamines by reversed phase liquid chromatography with electrospray tandem mass spectrometry. *J. Pharm. Biomed. Anal.* 45 (2007):625–634.
74. J. A. Byun, M. H. Choi, M. H. Moon, G. Kong, and B. C. Chung, Serum polyamines in pre- and post-operative patients with breast cancer corrected by menopausal status. *Cancer Letters* 273 (2009):300–304.
75. N.-N. Fu, H.-S. Zhang, M. Ma, and H. Wang, Quantification of polyamines in human erythrocytes using a new near-infrared cyanine 1-(-succinimidyl-hexanoate)-10-methyl-3,3,30,30 tetramethylindocarbocyanine-5,50-disulfonate potassium with CE-LIF detection. *Electrophoresis* 28 (2007): 822–829.
76. G. Liu, J. Chen, and Y. Ma, Simultaneous determination of catecholamines and polyamines in PC-12 cell extracts by micellar electrokinetic capillary chromatography with ultraviolet absorbance detection. *Journal of Chromatography B* 805 (2004):281–288.
77. R. L. H. Clements, A. Holt, E. S. Gordon, K. G. Todd, and G. B. Baker, Determination of rat hepatic polyamines by electron-capture gas chromatography. *Journal of Pharmacological and Toxicological Methods* 50 (1) (2004):35–39.
78. S. H. Lee, Y. J. Yang, K. M. Kim, and B. C. Chung, Altered urinary profiles of polyamines and endogenous steroids in patients

with benign cervical disease and cervical cancer. *Cancer Letters* 201 (2003):121–131.

79. L. Li, K. Harab, J. Liua, Y. Yua, L. Gaoa, Y. Wanga, and Y. Wang, Rapid and simultaneous determination of hair polyamines as N-heptafluorobutyryl derivatives by gas chromatography–mass spectrometry. *Journal of Chromatography B* 876 (2008): 257–260.
80. M. J. Paik, D. Kuon , J. Cho, and K.-R. Kim, Altered urinary polyamine patterns of cancer patients under acupuncture therapy. *Amino Acids.* 37 (2) (2008):407–413.
81. H. M. Woo, K. M. Kim, M. H. Choi, B. H. Jung, J. Lee, G. Kong, S. J. Nam, S. Kim, S. W. Bai, and B. C. Chung, Mass spectrometry based metabolomic approaches in urinary biomarker study of women's cancers. *Clinica Chimica Acta* 400 (2009):63–69.