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Diacetylated Derivatives of Spermine and Spermidine as Novel Promising Tumor Markers

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N^1,N^{12}-diacetylspermine (DiAcSpm) and N^1,N^8-diacetylspermidine (DiAcSpd) are minor components of human urinary polyamine to which little attention has been paid until recently. HPLC analysis of urinary polyamines has revealed 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. Remission was usually accompanied by recovery of urinary diacetylpolyamines to the normal level. DiAcSpm was more sensitive than CEA for detecting colorectal cancer patients, while DiAcSpd was highly specific for malignant conditions in that the excretion of the latter was scarcely elevated in cases of benign urogenital diseases. An ELISA procedure for rapid determination of DiAcSpm was developed to promote the clinical application of these new tumor markers, and subsequent studies indicated that DiAcSpm was elevated in 60% of colorectal cancer patients at early stages (stage 0 + I), whereas only 10% of these patients were CEA-positive. DiAcSpm may also be useful as a follow-up marker that is efficient for detecting recurrence and sensitive to changes in the clinical condition of patients. The evidence accumulated so far indicates that DiAcSpm and DiAcSpd are promising novel tumor markers. They deserve more intensive studies, including studies of their biochemistry and metabolism.

Key words: cancer diagnosis, diacetylspermidine, diacetylspermine, tumor marker, urine.

Diacetylpolyamines, what are they?

N^1,N^{12}-Diacetylspermine (DiAcSpm) and N^1,N^8-diacetylspermidine (DiAcSpd) are the least studied among the polyamine species that occur in human urine. They have a number of attractive features as diagnostic and prognostic indicators of malignant states, but unfortunately were discovered too late (*1*, *2*), only after the big tide of research on polyamines as tumor markers had almost ebbed away by the end of 1980's. For this reason they have failed so far to gain due interest even among clinical chemists involved in tumor marker study. This article aims to summarize the current status of diacetylpolyamine study, so that a wide audience can properly recognize the potential usefulness of this tumor marker.

Polyamines are alkylamines with multiple amino groups and are ubiquitous among both eukaryotic and prokaryotic cells. Putrescine, cadaverine, spermidine, spermine, and their acetylated derivatives are among most abundant organic polycations in the human body and are thought to fulfill multiple functions in biological processes (for review, see Refs. *3* and *4*). *Di*acetylated polyamine derivatives, N^1,N^8-diacetylspermidine (DiAcSpd) and N^1,N^{12}-diacetylspermine (DiAcSpm), which are featured in this article, are derivatives of spermidine and spermine, respectively, in which both of the primary amino groups are acetylated. In the past, these diacetylated derivatives did not receive much research attention since they were not detected by conventional methods, as explained later in this article.

Polyamines and their metabolism are supposed to be intimately involved in the process of cell multiplication and its regulation, although the details of their functions have not been fully elucidated in spite of extensive studies (*5*, *6*). Thus, exogenously added polyamines are indispensable for mutant cells defective in polyamine synthesis to grow in culture, and overexpression of ornithine decarboxylase, the key enzyme in polyamine biosynthesis, led to transformation of NIH 3T3 cells (*7*). Rapidly growing tissues usually have active polyamine synthesizing systems and contain large amounts of polyamines. Polyamine excretion in the urine may rise when such tissues are present in the body, as a result of either increased secretion from the proliferating cells themselves, or of release from dead cells that increase in number as a consequence of the active replacement of cells in growing tissues. In fact, Russell (*8*) reported in 1971 that the amount of polyamines excreted in urine was higher in patients with cancer than in healthy persons. This evoked a surge of studies on polyamine analysis that were intended to answer the question of whether the level of polyamines in urine could serve as indicators of malignant diseases.

There seemed to be sound reasons for expecting an increased excretion of polyamines in urine as a consequence of uncontrolled proliferation of cancer cells,

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because of the close relationship between cell growth and polyamine metabolism as described above, but the results obtained were, on the whole, rather disappointing. It was once claimed that the amount of N^1-acetylspermidine (N^1-AcSpd) and the ratio of N^1-AcSpd to N^8-acetylspermidine (N^8-AcSpd) were increased in cases of malignant diseases and that these polyamines might serve as tumor markers (*9*, *10*). However, the performance of these indicators was too poor for practical application because there were too many false negative as well as false positive cases due to large individual variations in polyamine excretion, and also because benign disorders were often found to be associated with considerable increases in urinary polyamine levels. The accumulated evidence, therefore, seemed to indicate that *free* and *mono*acetylated polyamines in urine were of little practical use as tumor markers, even though their average urinary levels are certainly higher in patients with cancer than in healthy persons (*11*). The tide of polyamine research as tumor markers was thus waning when we encountered the *di*acetylated derivatives of spermidine and spermine, namely, DiAcSpd and DiAcSpm (*1*).

At this point, a brief introductory description of diacetylpolyamines would be pertinent to convince the reader that they do have attractive features as tumor markers that are distinct from those of monoacetylpolyamines. The latter had been extensively studied so far, but were finally shown not to have practical value as tumor markers. Polyamines are excreted in human urine mainly as monoacetylpolyamines. Acetylputrescine (AcPut) is the most abundant urinary polyamine, constituting 43% of total polyamines, followed by acetylcadaverine, N^1-AcSpd and N^8-AcSpd in decreasing order. Diacetylpolyamines represent minor polyamine species in human urine. The average amounts of DiAcSpd and DiAcSpm are only approximately 1.4 and 0.46%, respectively, of total polyamines, but their CV values are very small, taking their low content into account (*1*). This indicates that variation of these diacetylpolyamines in urine is very small from one individual to another, and may imply that they are secreted in a highly controlled manner.

The amounts of individual polyamine components in urine were compared among healthy persons and patients with benign or malignant urogenital diseases (Fig. 1). The amounts of total polyamines, acetylputrescine and N^8-AcSpd were not significantly increased in a number of cancer patients as compared to those in healthy controls, while N^1-AcSpd was often elevated substantially even in cases of benign diseases. This is consistent with the orthodox judgement about urinary polyamines that they cannot be useful as practical tumor markers (*12*, *11*). In contrast, DiAcSpm was sensitive for detecting cancer patients in that it increased markedly in about 80% of patients with cancer, reaching as high as 150 times the normal limits, while DiAcSpd was highly specific for cancer in that it was elevated only marginally in cases of benign diseases (*2*). This strongly suggested that *di*acetylpolyamines but *not* monoacetylpolyamines would serve as novel tumor markers, and these early observations prompted us to perform further studies of diacetylpolyamines.

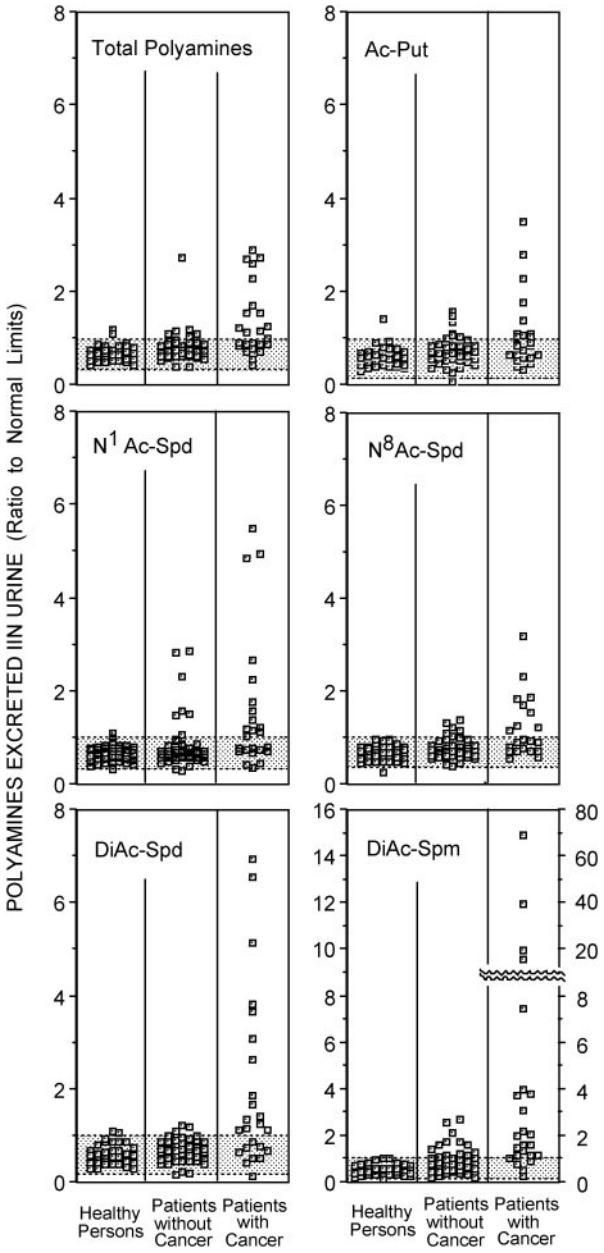

Fig. 1. **Polyamines excreted in urine of healthy persons and patients with benign or malignant urogenital diseases.** Shaded areas represent the mean ± 2 SD for healthy persons. Mean (SD) values (μmol/g creatinine) for healthy persons are as follows: total polyamines 22.2 (6.1), acetylputrescine (Ac-Put) 9.6 (3.5), N^1-acetylspermidine (N^1Ac-Spd) 2.7 (0.8), N^8-acetylspermidine (N^8Ac-Spd) 2.4 (0.6), diacetylspermidine (DiAc-Spd) 0.30 (0.11), diacetylspermine (DiAc-Spm) 0.10 (0.047). (With permission, Sugimoto *et al.*, *J. Cancer Res. Clin. Oncol.*, **121**, 317, Copyright 1995: Springer Verlag)

Simultaneous detection and determination of polyamine components including diacetylpolyamines

Several methods were reported so far in the literature to determine the amounts of diacetylpolyamines in biological fluids and tissues. It should be noted that conventional

polyamine detection systems utilizing primary amino groups for post-column derivatization of polyamine components (*13*) are unsuitable for detection of diacetylpolyamines which lack primary amino groups.

Gas chromatography with nitrogen phosphorus detection was used by van den Berg *et al*. to detect DiAcSpm in five arbitrarily selected patients with stage III and IV non-Hodgkin's lymphoma (*14*). This was the first report that described the occurrence of DiAcSpm in human body fluid, but the correlation between DiAcSpm and cancer was not pursued further by the Dutch group. Their analysis failed to detect DiAcSpm in the urine of healthy persons and they did not describe the occurrence of DiAcSpd either.

Diacetylpolyamines can also be determined by fractionation with HPLC connected to an enzymatic detection system (*1*). In this system, urine samples pretreated with a cation exchange resin to remove interfering substances were applied to a polymer-based C18-column and polyamine components were eluted with phosphate buffer. Polyamines separated by HPLC were introduced into an enzyme reactor composed of three successive small columns of immobilized enzymes, namely acylpolyamine deacylase (DAL), polyamine oxidase (PAO) and putrescine oxidase (PUO). In this enzyme reactor, acetylated polyamines were first deacetylated and then oxidized to generate H_2O_2 that was then quantitated by an electrochemical detector. In this way, all the polyamine components naturally occurring in human urine may be determined precisely and simultaneously (*15*). This procedure was sensitive enough to detect DiAcSpm and DiAcSpd in the urine of healthy persons, and further led us to the finding that these diacetylated polyamine species, but not monoacetylated polyamines, are frequently and sometimes markedly increased in cancer patients, as described in the preceding section. Unfortunately, however, these enzymes are now unavailable commercially, for companies that used to distribute these enzymes have ceased their production. More recently, a procedure was developed that fractionates dansylated polyamines by HPLC using a C18 column (*16*, *17*). This procedure allowed the fractionation and detection of diacetylated polyamines. Polyamine components including DiAcSpm may also be determined by ionspray ionization-mass spectrometry (IS-MS) after heptafluorobutyryl derivatization (*18*).

Development of diacetylpolyamine-specific antibodies and immunochemical determination of diacetylpolyamines

Simultaneous determination of urinary polyamines clearly indicated that only diacetylpolyamines (DiAcSpm and DiAcSpm), but not monoacetylpolyamines, had very attractive features that deserved further examination regarding whether they were useful as novel tumor markers. To open pathways leading to clinical applications of diacetylpolyamines, it was necessary to devise a reliable procedure for the determination of DiAcSpm and DiAcSpd that is simpler and more convenient than HPLC analysis, since the latter is too time-consuming to be useful in clinical practice.

An enzyme-linked immunosorbent assay (ELISA) procedure involving specific antibodies was thus developed. Antibodies used in the immunochemical determination of DiAcSpm needed to be highly specific for this component. Human urine usually contains N^1-AcSpd in amounts that are in approximately 30-fold molar excess of DiAcSpm (*1*). This implies that the cross-reactivity of N^1-AcSpd with DiAcSpm should be less than 0.1% in order to keep the overestimation of urinary DiAcSpm due to contaminating N^1-AcSpd less than 10%. Polyclonal antibodies were raised in rabbits by immunization with a carefully designed hapten-BSA conjugate. In this conjugate monoacetylspermine (AcSpm) was coupled to the protein carrier *via* a cross-linker, γ-maleimidobutyryloxysuccinimide, to form an acylamide linkage between the primary amino group of AcSpm and the butyryl group of the cross-linker (*19*). This gave a hapten structure that closely mimics the structure of DiAcSpm. The rabbit antiserum obtained through immunization with this antigen showed much better preference for DiAcSpm as compared to antipolyamine antibodies reported in the literature, with cross-reactivity with N^1-AcSpd of about 5–6%, but still failed to meet the requirement for the specificity of an antibody to be used in the determination of urinary DiAcSpm. Antibodies highly specific for DiAcSpm, with cross-reactivity with N^1-AcSpd as low as 0.03%, were eventually obtained from this antiserum through several steps of affinity purification (*19*). In the final step of this purification, DiAcSpm-specific antibodies were adsorbed on an affinity resin containing DiAcSpm-mimicking ligands, and the fraction of the adsorbed antibodies that remained bound to the affinity resin after elution with N^1-AcSpd was recovered as the final preparation of DiAcSpm-specific antibody.

A competitive ELISA system for DiAcSpm was developed using this antibody preparation. The system was sensitive enough to allow accurate determination of DiAcSpm at concentrations that are usually excreted in healthy human urine. DiAcSpm concentrations in urine determined by this procedure correlated very well with those obtained by HPLC analysis (*19*). The development of more convenient procedures for determining DiAcSpm using specific antibodies which are adapted for automatic analytical apparatus is currently being attempted.

Monoclonal antibodies specific for diacetylpolyamines were also reported, but their specificity did not seem to be sufficient for use in the determination of urinary DiAcSpm and DiAcSpd (*20*, *21*). Very recently, success in the production of a monoclonal anti-DiAcSpm antibody that was highly specific for DiAcSpm was announced by a company.

DiAcSpd-specific polyclonal antibodies were also prepared (*22*), but procedures for determining the urinary DiAcSpd level using these antibodies have not been established yet.

Sensitivity of DiAcSpm as a marker for various cancers

Diurnal variation of DiAcSpm excretion into the urine tends to parallel that of creatinine (Fujie, N. and Kawakita, M., unpublished observation). The daily excretion of DiAcSpm in the urine of individuals may thus be compared in terms of the amount of DiAcSpm excreted per g creatinine, using arbitrarily collected spot urine samples. The amount of DiAcSpm excreted in the urine of healthy

persons was small as compared with that of monoacetylated polyamine derivatives, and was distributed in a very narrow range, with a mean ± SD value of 0.15 ± 0.05 μmol/g creatinine for 52 apparently healthy volunteers (*1*), but was frequently increased in patients with neoplastic diseases.

Thus, the sensitivity of DiAcSpm (100 × number of true positives/total patients) in the 248 colon cancer patients examined was 75.8% in reference to 0.25 μmol/g creatinine (mean + 2 SD of healthy persons) as a cutoff value, whereas the specificity of DiAcSpm (100 × number of true negatives/total subjects in a control group) was 96% for the 52 apparently healthy controls and 77% for the 51 patients with benign gastrointestinal diseases (*23*). Patients with active inflammatory symptoms, including those with acute appendicitis and acute cholecystitis and those in the active phase of inflammatory bowel diseases tended to give DiAcSpm values above the cutoff level (*23*), but these patients could be easily identified by their characteristic symptoms. Besides these easily discernible patients, those who were being treated for benign diseases including adenomas excreted similar amounts of DiAcSpm as healthy persons.

The sensitivity of DiAcSpm for colon cancer (75.8%) was much higher than that of tumor markers which are currently in clinical use, such as serum CEA (39.5% with 5 ng/ml as a cutoff value, $P < 0.0001$) and CA19-9 (14.1% with 37 units/ml as a cutoff value, $P < 0.0001$) (*23*). Urinary DiAcSpm may thus be considered a novel high-performance tumor marker for colon cancers that can be favorably compared with these tumor markers.

Urinary DiAcSpm levels were examined for patients with various cancers. Earlier analysis using an HPLC procedure indicated that DiAcSpm was elevated frequently and markedly in urogenital malignancies, including prostate cancer, testicular cancer, renal cancer and renal pelvic cancer, with a very low false-negative incidence (*2*). DiAcSpm was also reported to be increased in patients with leukemia (*14*, *24*). More recently, DiAcSpm levels in pancreatobiliary adenocarcinoma were shown to be increased significantly ($P < 0.001$) as compared to those in a control group consisting mainly of patients with benign inflammatory diseases and adenomas. The sensitivity of urinary DiAcSpm for malignant conditions was 75%, which was higher than that of CEA (44%, $P = 0.044$) and the same as that of CA19-9 (75%) (*25*). Analysis of urine samples from 83 breast cancer patients further indicated that DiAcSpm was above the cutoff value in 60.2% of the patients. The sensitivity of DiAcSpm for breast cancer was significantly higher than the sensitivities of serum CEA (37.3%, $P = 0.0032$) and CA15-3 (37.3%, $P = 0.0032$) (*23*). Patients with hepatocellular carcinoma also show a high incidence of elevated urinary DiAcSpm (*26*). Thus, urinary DiAcSpm was increased more frequently than most tumor markers currently in clinical use in patients with various types of cancers. Our preliminary studies suggested that DiAcSpm in the urine was also increased in cases of brain tumors and lung cancers.

These results strongly suggest that DiAcSpm may be useful to detect a wide variety of neoplastic diseases. It is quite reasonable that various cancers are associated with increased DiAcSpm excretion in the urine, because polyamine metabolism is activated in association with various types of cell proliferation.

Sensitivity of DiAcSpm for early-stage cancers

One of the most prominent features of DiAcSpm is that it can detect early-stage colon, breast and other cancers in patients (*23*). The proportion of positives with respect to known tumor markers is usually very low for early-stage cancers. For instance, when the proportion of positives with respect to a given marker was compared among colon cancer patients grouped according to tumor stage, the serum CEA values were above the cutoff value in only 10% of stage 0 ($n = 20$) and stage I ($n = 40$) patients. On the other hand, urinary DiAcSpm was positive in 60% of these early stage colon cancer patients. The difference in the sensitivity between DiAcSpm and CEA was significant for both stages ($P = 0.002$ for stage 0 and $P < 0.0001$ for stage I) (Table 1). The sensitivity of DiAcSpm for detecting early-stage colon cancer was comparable to that of the fecal occult blood test, which is widely used in screening for colon cancer.

Similarly, the sensitivity of DiAcSpm for early-stage breast cancer (stages I and II; 28.1%) was significantly higher than those of CEA (3.1%; $P = 0.018$) and CA15–3 (0%; $P = 0.001$) (*23*). Although the sensitivity of DiAcSpm for early-stage breast cancer was not as high as that for early-stage colon cancer, it would certainly be useful in the early diagnosis of breast cancer, since the sensitivity of currently available markers such as CEA and CA15-3 is negligibly low for breast cancer at earlier stages.

DiAcSpm and CA19-9 showed comparable sensitivity for stage IIb pancreatobiliary carcinoma cases, being positive in 3 and 4 out of 6 cases, respectively, whereas CEA was positive in none of these cases (*25*). It seems likely that the

Table 1. Colon cancer stage and tumor marker levels.

Stage	*n*	DiAcSpm			CEA			CA19-9		
		Positive	Negative	Sensitivity (%)	Positive	Negative	Sensitivity (%)	Positive	Negative	Sensitivity (%)
0	20	12	8	60.0[1]	2	18	10.0	1	19	5.0
I	40	25	15	62.5[2]	4	36	10.0	2	38	5.0
II	60	43	17	71.7[3]	25	35	41.7	3	57	5.0
III	107	89	18	83.2[2]	53	54	50.5	24	83	22.4
IV	21	19	2	90.5[4]	14	7	66.7	5	16	23.8
Total	248	188	60	75.8[2]	98	150	39.5	35	213	14.1

[1] $p = 0.002$ compared to CEA and $p = 0.0004$ compared to CA19-9. [2] $p < 0.0001$ compared to CEA and CA19-9. [3] $p = 0.0009$ compared to CEA and $p < 0.0001$ compared to CA19-9. [4] $p < 0.0001$ compared to CA19-9. (With permission, Hiramatsu *et al.*, *Clin. Cancer Res.*, **11**, 2986, Copyright 2005: Am. Assoc. Cancer Res.)

urinary DiAcSpm level would also be sensitive for malignant conditions at earlier stages which have not been examined so far.

It should be noted that DiAcSpm was not usually increased in cases of adenoma, thus allowing us to discriminate between malignant and nonmalignant tumors (*23*, *25*). The molecular basis for this distinction between malignant and nonmalignant tumors remains to be elucidated. Early diagnosis of malignant conditions is of great importance in cancer therapy, because cancers can often be cured if detected early enough, owing to the recent progress in cancer therapy. The availability of a tumor marker that would enable us to find cancers in patients at an earlier stage than is possible using currently available methods would contribute much to reducing fatal cases of cancer. DiAcSpm seems a promising candidate for such a marker.

DiAcSpm as an indicator of effectiveness of treatments and of recurrence

The urinary level of DiAcSpm and DiAcSpd in patients with prostate cancer and testicular cancer tended to be lowered in response to treatment, when effective, and the diacetylpolyamine level after treatment was correlated with the prognosis of a patient (*27*). Patients fell into 3 groups in terms of the change in urinary DiAcSpm and DiAcSpd level in response to treatment. In the first group, a precipitous decrease of both DiAcSpm and DiAcSpd to the normal level was noted. The physical conditions of the patients in this group were markedly improved as a result of the treatment, and they were usually free from recurrence of cancer for 3 years. In the second group of patients, in contrast, DiAcSpm and DiAcSpd remained much higher than the normal level even when the patients were clinically judged to be in a state of partial remission. These patients suffered from recurrence within 6 months with a concomitant elevation of urinary diacetylpolyamine level, and eventually died from prostate cancer. In some patients (the third group of patients) the response of the urinary diacetylpolyamine level to treatment for cancer was not clear-cut, with the DiAcSpm level remaining for some time in a range slightly higher than normal. Further studies indicated that patients in this group were not threatened by an immediate risk of recurrence, and that they usually tended to achieve complete remission in 3 years with concomitant recovery of a normal DiAcSpm level (*27*).

The sensitivity of urinary DiAcSpm for the detection of recurrence of pancreatobiliary carcinoma was slightly higher than that of serum CEA and CA19-9, while its specificity was slightly lower than that of these serum markers (*25*).

These results indicate that DiAcSpm and DiAcSpd in the urine may be useful in assessing the effectiveness of treatment, and the diacetylpolyamine level during the follow-up period of patients in remission may serve as a sensitive indicator of the recurrence of cancer. It is also important that the level of these diacetylpolyamines after treatment may be informative of the prognosis of patients with prostate cancer and testicular cancer. However, the number of cases as well as types of cancers so far analyzed in these respects has been quite limited. It is necessary to accumulate enough information to verify the above-noted characteristics of DiAcSpm and DiAcSpd. Preliminary examination of colorectal cancer and brain tumor cases seems to support the usefulness of urinary DiAcSpm as a follow-up marker and prognostic indicator (Hiramatsu *et al*., unpublished observation).

Possible metabolic events leading to increase in excretion of diacetylpolyamines in the urine

The metabolism of DiAcSpm and DiAcSpd is not fully understood yet. Spermidine/spermine N^1-acetyltransferase (SSAT) catalyzes the acetylation of the N^1-amino groups of spermidine, spemine and N^1-acetylspermine, but not of N^8-acetylspemidine *in vitro* (*28*, *29*). At the cellular level, conditional overexpression of SSAT in MCF-7 human breast cancer cells led to an accumulation of high levels N^1-AcSpd, *N*-AcSpm and DiAcSpm. Concomitantly, intracellular putrescine and spermidine pools were lowered, and cell growth was inhibited (*17*). Chen et al. also observed an increase in DiAcSpm level associated with the induction of SSAT in SK-MEL-28 human melanoma cell line treated with diethylnorspermine (DENSPM) (*30*).

A gradual increase in the SSAT activity was noted during the tumor growth of Yoshida AH-130 ascites hepatoma cells (*31*) and an increase in N^1-AcSpd concentration was observed in many kinds of cancers (*32*). Production of DiAcSpm would also be increased under such situations, although an elevated level of intracellular DiAcSpm has not been demonstrated so far. The induction of SSAT may stimulate oxidative catabolism of polyamines *via* polyamine oxidase on the one hand, and excretion of acetylated polyamines on the other, together leading to lowering the intracellular polyamine pools (*17*). This may represent a homeostatic mechanism to lower intracellular polyamines under conditions of sustained high levels of ornithine decarboxylase in cancer cells. It is possible that increased excretion of acetylated polyamines, including DiAcSpm, from cancer cells may be a consequence of a feedback response of rapidly growing cells in the tissues to down-regulate the elevated cellular polyamine levels to suppress their uncontrolled growth. On the other hand, enhanced polyamine catabolism seemed positively correlated with carcinogenesis in some cases (*33*).

We do not fully understand yet how an elevated urinary polyamine level may actually be attained in patients with cancer. In spite of the fact that the SSAT activity tends to be increased in cancer cells, it is still unclear whether the increase in the DiAcSpm level in the urine of patients with cancer could be explained directly by an increase in the amount of DiAcSpm excreted as such from cancer cells or tissues themselves. It is also possible that active polyamine metabolism in rapidly growing tissues including cancer tissues leads to an elevation of the whole polyamine level in the circulation and that the acetylation of circulating polyamines takes place in other tissues or organs which remain to be identified, leading to an elevated level of acetylated polyamine derivatives, including DiAcSpm. It is worth mentioning here that the erythrocyte spermidine and spermine levels, which would reflect the activity of whole-body polyamine metabolism, were reported to be correlated well with the tumor stage of prosthetic carcinoma patients and tended to

increase in metastatic and hormonal escape patients (*34*, *35*).

In considering the polyamine level in the urine we should not ignore the reabsorption of polyamines at the renal brush border. Polyamines excreted from various organs and tissues into the circulation are filtered through the glomerular basement membrane in the kidney, but a significant portion of monoacetylpolyamines as well as unconjugated polyamines are soon reabsorbed from the glomerular filtrate into tubular cells through polyamine transport systems, and converted to free polyamines by cellular polyamine oxidase to be reutilized in the body. Although polyamine transporter genes in mammals have not yet been identified, the properties of polyamine transport systems in renal epithelia can be examined by using polarized monolayers of cells derived from renal proximal tubules such as porcine LLC-PK_1 cells (*36*, *37*). Measurement of polyamine uptake using this model system indicated that monoacetylpolyamines were actively transported into the cells from the apical surfaces. DiAcSpm did not inhibit any polyamine transport system expressed in the apical membranes of the cell monolayers, nor was it incorporated into the cells (*38*). This implies that the renal reabasorption route is available for monoacetylpolyamines, but not for DiAcSpm. It is therefore likely that DiAcSpm excreted from cells in the body is recovered in the urine without significant loss, while the amount of urinary monoacetylpolyamies decreases by an unknown amount compared to that originally excreted from the cells due to renal reabsorption and reutilization. This may at least partially explain why the urinary level of DiAcSpm reflects the presence of cancer in the body with high sensitivity and at early clinical stages, while the urinary levels of monoacetylpolyamines do not (*38*).

As described in a previous section, DiAcSpd behaved differently from DiAcSpm. It was not as sensitive as DiAcSpm for detection of urogenital malignancies, but was highly specific for malignant conditions in that the DiAcSpd level in patients with benign urogenital diseases was very similar to that in healthy persons (see Fig. 1) (*2*). It is worth noting that the excretion of DiAcSpm was tremendously increased in the urine of pregnant women (*39*), while that of DiAcSpd was not (Hiramatsu et al., unpublished observation). This implies that the induction of the enzyme activity responsible for the synthesis of DiAcSpd may be closely related to malignant conditions. Unfortunately, however, the molecular identity of the acetyltransferase responsible for the synthesis of DiAcSpd is not yet known. The identification, characterization and regulation of this enzyme would be of great interest. It will also be important to examine the behavior of urinary DiAcSpd in various malignant conditions other than urogenital malignancies. A precise and convenient procedure for determination of DiAcSpd should be developed for this purpose. The studies on DiAcSpd as a tumor marker have lagged behind those on DiAcSpm for various reasons, but it certainly deserves more attention than so far paid to it.

Future perspectives

The activation of polyamine metabolism is closely related to active cell proliferation. The increased excretion of DiAcSpm and DiAcSpd in cancer patients so far examined is clearly related to this phenomenon. It is therefore highly likely that urinary diacetylpolyamine levels are increased in various types of cancers and that they could serve as novel tumor markers applicable to a wide variety of cancers, since active and uncontrolled cell proliferation is a common characteristic of cancer cells. Intensive examination of urinary diacetylpolyamine levels in cancers which have not been fully examined yet would be worthwhile.

Early detection of patients with cancer would greatly improve the efficacy of treatment and help in reducing the fatal cases of cancers. One of the prominent features of DiAcSpm may be that it is highly sensitive for early-stage cancers, including colorectal cancer, breast cancer and pancreatobiliary cancer (*23*, *25*). On the other hand, its sensitivity for hepatocellular carcinoma at earlier stages may not be higher than that of other markers such as AFP or PIVKA II, although the number of cases analyzed has been rather limited so far (*26*). The usefulness of DiAcSpm as a sensitive indicator of cancers at earlier stages should be carefully examined. Urine samples are easily obtained noninvasively from individuals and are thus suitable test materials in health examinations. The use of urinary DiAcSpm as an item in public health examination for screening of potential cancer-bearing patients, perhaps in combination with other markers such as the fecal occult blood test, may considerably improve the efficiency of early colorectal cancer detection. In this context, whether the results of the fecal occult blood test and the urinary DiAcSpm value are correlated is a very intriguing question.

The increase of DiAcSpm in the urine does not specify the location of a cancer tissue in the body. It should be noted, however, that specificity to a particular type of cancer is unnecessary when a tumor marker is considered as an item for screening of unspecified cancer patients, as a follow-up marker, and as a prognostic indicator. Such specificity may rather restrict the applicability of the marker to a narrow range of diseases. Results accumulated so far indicate that DiAcSpm may be useful as a tumor marker in various respects, as noted above. The characteristic of DiAcSpm that it is not tumor type specific would then be considered as one of its attractive features. A follow-up marker that is efficient in detecting recurrence and correlates well with the prognosis of a patient would be invaluable in improving the QOL of the patient. DiAcSpm may be a candidate for such an attractive follow-up marker.

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