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Diagnostic approach to breast cancer patients based on target metabolomics in saliva by liquid chromatography with tandem mass spectrometry

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ABSTRACT

Background: Breast cancer is one of the most fearful diseases due to its increasing worldwide prevalence. A number of screening tests has been employed including clinical examinations and mammography. However, another screening method, which is a simple, not embarrassing, and low cost, is highly desired. Based on these findings, we are currently investigating the determination of polyamines including their acetylated structures for the diagnosis of breast cancer patients. We established a diagnostic approach to breast cancer patients based on the ratios of polyamines in saliva by a UPLC-MS/MS analysis.
Methods: Twelve polyamines including their acetylated form were labeled with DBD-F, separated by a reversed-phase chromatography and detected by a Xevo TQ-S tandem mass spectrometer.
Results: Eight polyamines (e.g., SPM, CAD, Ac-SPM, N1-Ac-SPD, N8-Ac-SPD) strongly correlated with the cancer patients. A simple 1-order equation was developed for the discrimination of the breast cancer patients and healthy persons ($Y = 0.5X_{SPM} - 3X_{Ac\text{-}SPM} - 0.15X_{SPD} - 3.5X_{N8\text{-}Ac\text{-}SPD} + 0.5X_{N1\text{-}Ac\text{-}SPD} + 0.04X_{CAD}$). The concordance rate of the breast cancer patients and the healthy persons by the equation was 88% and 76% on the training set, respectively, whereas those on the validation set was both 88%. The score Y in the equation tended to correlate with the cancer stage of the patients and increased with the more serious conditions. The determination of polyamines in the saliva after the cancer patient operations was also performed to identify the concentration change before and after the surgical treatment. The discriminant analysis using 6 polyamines (i.e., N8-Ac-SPD, N1-Ac-SPD, CAD, DAc-SPD, PUT, and Ac-PUT), which were the most influenced molecules derived from the ROC analysis, was performed using the relative percentage. Both the sensitivity and specificity indicated nearly 80% from the ROC analysis result using the ratio of N8-Ac-SPD/(N1-Ac-SPD + N8-Ac-SPD).
Conclusion: The discrimination equation appears to be useful for the diagnosis of breast cancer patients. Furthermore, the ratio of N8-Ac-SPD/(N1-Ac-SPD + N8-Ac-SPD) may be adopted as an index of the health status after the surgical treatment.

1. Introduction

Breast cancer is one of the most fearful diseases due to its increasing worldwide prevalence, not only in European-Americans, but also in Asian countries [1]. Its prevalence is due to not only intrinsic genetic specificity, but also modern lifestyles. Most types of breast cancers are easy to diagnose by microscopic analysis of the biopsy. While screening techniques are useful in determining the possibility of cancer, further testing is necessary to confirm the disease state. A number of screening tests have been employed including clinical examinations and mammography. However, women struggle against these tests because of the embarrassment, waste of time, and high expense. Consequently, another screening method, which is a simple, not embarrassing, and low cost, is highly desired. There are many different tumor markers, which are indicative of a particular disease process, and they are used in oncology to help detect the presence of cancer. Several tumor markers, such as CEA and CA15-3, are known to increase in breast cancer patients [2]. However, the elevated level of the tumor markers can also be due to other causes. These markers are high-molecular mass substances such as glycoproteins and enzymes.

As relatively small molecules, polyamines have been significantly associated with rapid tumor growth due to their biosynthesis and accumulation [3]. Therefore, the concentrations of the polyamines,

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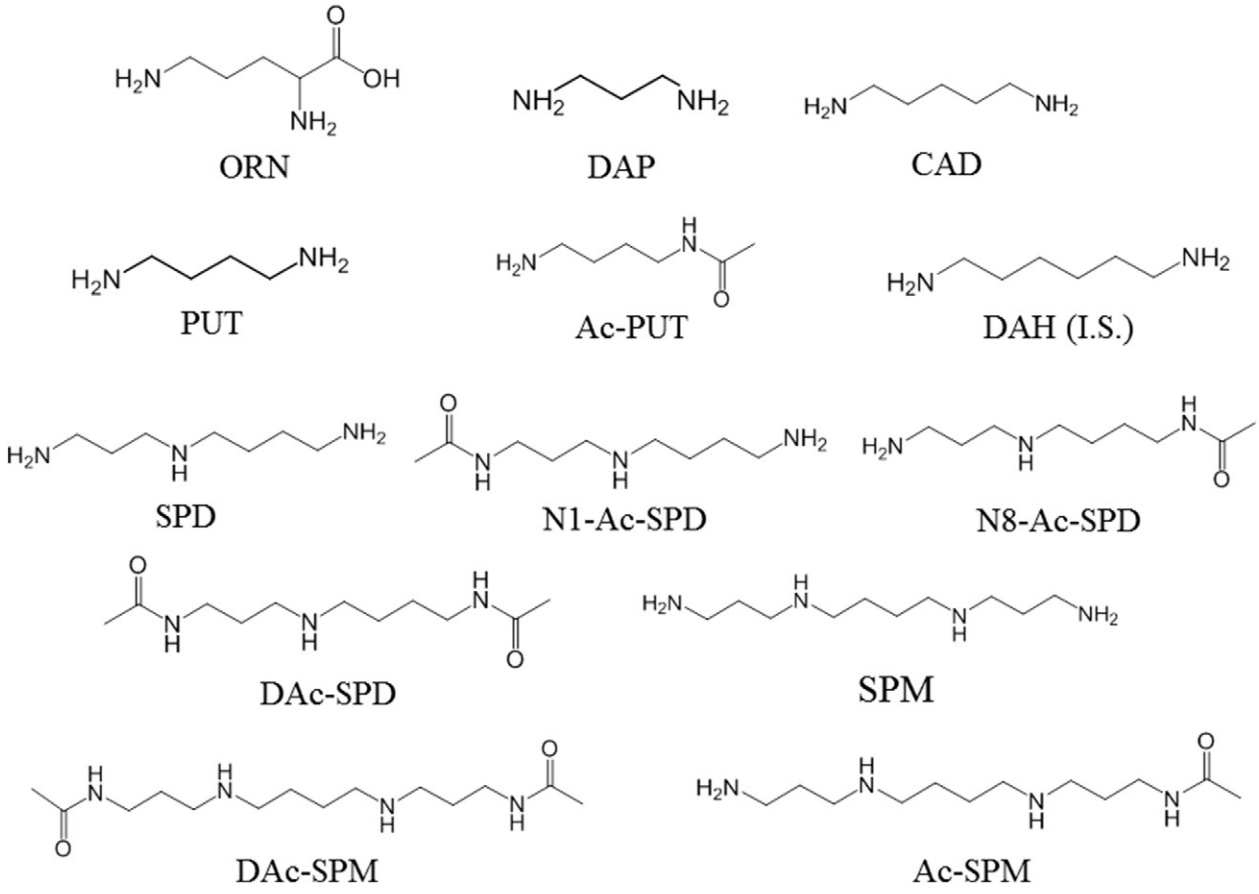
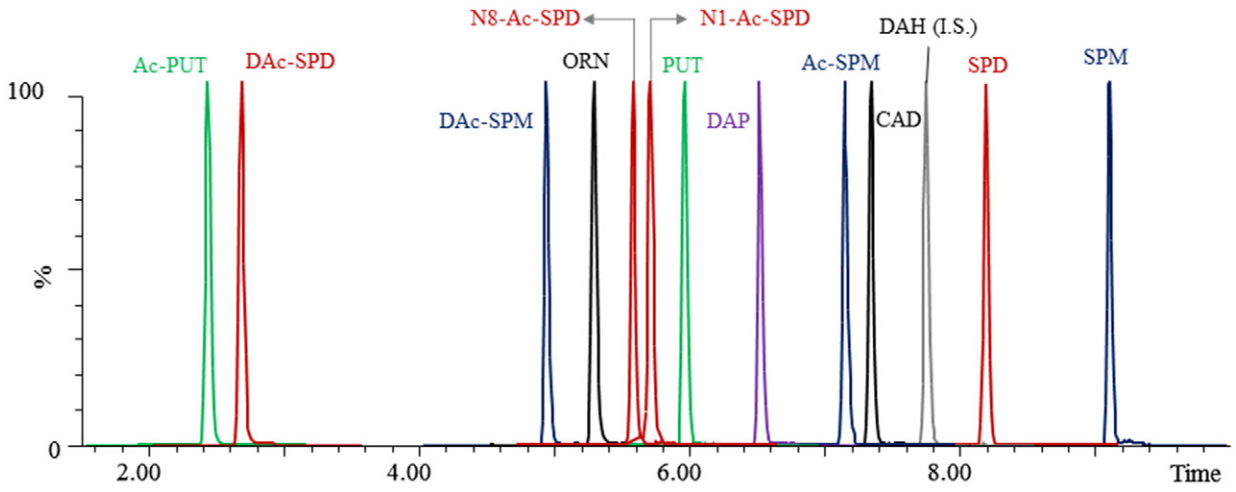


Fig. 1. Structures of polyamines and I.S. (DAH).



UPLC condition	
Column	ACQUITY UPLC™ BEHC18 column (100 mm × 2.1 mm i.d., 1.7 μm)
Mobile Phase	A 0.1% HCOOH in Water B 0.1% HCOOH in MeCN
Gradient of B%	20-60-90% (0-8-10 min)
Column Temp.	40 ℃
Injection volume	5 μL

MS/MS condition	
Ion Mode	ESI positive
Capillary Voltage	3.00 kV
Cone Voltage	50 V
Collision gas flow	0.15 mL /min
Collision energy	10-38 eV
Desolvasion Temp.	500℃

Fig. 2. SRM chromatograms of the DBD-polyamine derivatives.