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RESEARCH PAPER

SERS assessment of the cancer-specific methylation pattern of genomic DNA: towards the detection of acute myeloid leukemia in patients undergoing hematopoietic stem cell transplantation

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Abstract

In this label-free surface-enhanced Raman scattering (SERS) study of genomic DNA, we demonstrate that the cancer-specific DNA methylation pattern translates into specific spectral differences. Thus, DNA extracted from an acute myeloid leukemia (AML) cell line presented a decreased intensity of the 1005 cm^{-1} band of 5-methylcytosine compared to normal DNA, in line with the well-described hypomethylation of cancer DNA. The unique methylation pattern of cancer DNA also influences the DNA adsorption geometry, resulting in higher adenine SERS intensities for cancer DNA. The possibility of detecting cancer DNA based on its SERS spectrum was validated on peripheral blood genomic DNA samples from $n = 17$ AML patients and $n = 17$ control samples, yielding an overall classification of 82% based on the 1005 cm^{-1} band of 5-methylcytosine. By demonstrating the potential of SERS in assessing the methylation status in the case of real-life DNA samples, the study paves the way for novel methods of diagnosing cancer.

Keywords Acute myeloid leukemia · 5-Methylcytosine · SERS · DNA methylation · Epigenetics

Vlad Moisoiu and Andrei Stefancu contributed equally to this work.

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Introduction

Cancer DNA displays a unique pattern of methylation that differs both quantitatively and qualitatively from normal DNA. Compared to normal DNA, cancer DNA exhibits an overall decrease in the percentage of methylated cytosines [1]. Moreover, the methylated cytosine residues of cancer DNA are clustered in the so-called CpG islands that precede the genes, whereas other areas of the cancer DNA are almost devoid of methyl cytosines [2].

The unique methylation pattern of cancer DNA has been exploited for improving the diagnostic accuracy of detection methods such as next-generation sequencing (NGS) of circulating tumor DNA [3]. Recently, Sina et al. brought compelling evidence showing that the cancer-associated DNA methylation pattern is also responsible for an increase in the affinity of cancer DNA to gold surfaces, a feature which enabled its sensitive detection by electrochemical methods [4].

We started this study with the assumption that the increased affinity of cancer DNA to metal surfaces could also be exploited for detecting malignant DNA based on surface-enhanced Raman scattering (SERS), an analytic method which requires the adsorption of the analytes onto metal nanosubstrates [5, 6]. Raman spectroscopy is a type of vibrational spectroscopy which is based on the inelastic scattering of laser photons, providing information regarding the molecular structure of the sample. The inherently low sensitivity of Raman scattering can be improved significantly by SERS, a method which uses metal nanostructures for amplifying the Raman signal of molecules [7].

In this study, we developed an optimized protocol for promoting the chemisorption of DNA onto Ag nanoparticles and assess by SERS the methylation status of genomic DNA extracted from peripheral blood cells, as well as from cultured cell lines. The SERS substrate was represented by a chloride containing Ag nanoparticle colloidal solution, synthesized by reduction with hydroxylamine hydrochloride (hya-AgNPs). The hya-AgNPs were additionally activated with Ca^{2+} 5×10^{-4} M in order to promote the chemisorption of Cl^- onto the silver surface, which in turn forms DNA-specific SERS active sites [5, 6].

Given that changes in the cytosine methylation status of DNA are known to be an essential part of cancer progression, we focused our study on detecting by SERS the spectral differences that arise because of the unique pattern of methylation seen in cancer DNA. For this, we screened the SERS spectrum of cytosine and 5-methylcytosine for bands that are specific to the methylated cytosine. Next, we analyzed DNA samples extracted from normal cell line and a human acute myeloid leukemia (AML) cell line, with the aim of demonstrating that the cancer-associated hypomethylation can be evidenced by SERS even in genomic DNA samples. Finally, we validated the SERS-based cancer DNA detection strategy on real-life peripheral blood genomic DNA samples from healthy volunteers (n = 17) and patients diagnosed with AML (n = 17). Leukemia is a convenient disease for validating DNA-based liquid-biopsy tools, blood is the underlying tissue affected by the malignancy and because there is an extensive knowledge about the molecular biology mechanism behind cancer onset and progression. To the best of our knowledge, this study represents the first report concerning the detection of cancer-associated DNA methylation pattern in real-life samples.

Materials and methods

Silver nanoparticle synthesis The Ag nanoparticles obtained by reduction with hydroxylamine hydrochloride (hya-AgNPs) were prepared using the method introduced by Leopold and Lendl [7]. The synthesis protocol and the UV-vis spectrum of the colloidal hya-AgNPs are presented in the Electronic Supplementary Material (ESM Fig. S1). For all SERS experiments on DNA, the hya-AgNPs were activated with $Ca(NO_3)_2$ 5×10^{-4} M [5, 6]. The hya-AgNPs also contain Cl^- 2.4 mM, released during the synthesis reaction, which play a crucial role in mediating the adsorption of DNA onto the metal surface [5, 6].

Extraction of DNA from cells The cancer genomic DNA was extracted from a human immortalized AML cell line (THP-1) (American Type Culture Collection), while the normal DNA was extracted from immortalized human keratinocytes (HaCaT) (Thermo Fisher Scientific) using a solid phase extraction system (PureLink Genomic DNA mini kit, Thermo Fischer). THP-1 is an immortalized human monocytic cell line derived from a patient diagnosed with acute monocytic leukemia while HaCaT is an immortalized human keratinocyte cell line derived from human skin. Approximately 5×10^6 cells grown in standard cell culture conditions were detached using trypsin, centrifuged and then resuspended in 200 μl PBS. The cells were then incubated for 2 min at room temperature with 20 μl of Proteinase K and 20 μl RNase A. Next, the cells were incubated with 200 μl of PureLink™ Genomic Lysis/Binding Buffer for 10 min at 55 °C in order to promote protein digestion. The resulting solution was mixed with 200 μl ethanol, added to a PureLink™ Spin Column and then centrifuged at 10,000×g for 1 min at room temperature. The column was then washed by centrifugation at 10,000×g for 1 min at room temperature with two wash buffers (500 μl) provided in the kit. Finally, the DNA was eluted with 50 μl of PureLink™ Genomic Elution Buffer by centrifuging the column at 10,000×g for 1 min at room temperature.

Collection of peripheral blood genomic DNA Peripheral blood genomic DNA samples were collected from $n = 17$ controls and $n = 17$ AML patients undergoing hematopoietic stem cell transplantation at the Clinical Institute of Urology and Kidney Transplant, Cluj-Napoca. The average age of the AML patients was 47 ± 12 years while the average age of the control subjects was 40 ± 15 years. In both groups, the age had a parametric distribution (D'Agostino and Pearson omnibus normality test, $p > 0.05$) and the difference between the age of the two groups was not statistically different (two-tailed unpaired t test, $p > 0.05$). Five patients in the AML group and 7 subjects in the control group were females. The DNA samples were remnants from the HLA matching assay, and they represented medical waste. All samples were anonymized. The study was approved by the Ethics Committee of the Clinical Institute of Urology and Kidney Transplant, Cluj-Napoca and the experiments were conducted according to the principles of the Declaration of Helsinki.

The genomic DNA was extracted automatically from whole blood on EDTA 5% using the InnuPure C16 system (Analytik Jena) per manufacturer's protocol. The concentration and purity of DNA was determined automatically using NanoDrop 2000 (Thermo Scientific). The purity of the DNA was calculated based on the ratio between the absorbance at 260 and 280 nm (A260/A280). Concentration and purity of the DNA samples are presented in Table 1.

Acquisition of SERS spectra For the acquisition of SERS spectra of DNA, an InVia Raman (Renishaw) spectrometer was used, equipped with a doubled frequency Nd:YAG laser emitting at 532 nm, which was focused on the sample through a × 5 objective (Leica, NA 0.12).

For the SERS measurements of DNA extracted from peripheral blood and from cultured cell lines, 5 μl hya-AgNPs was mixed with 5 μl DNA. Afterwards, 0.5 μl $Ca(NO_3)_2$ (final concentration 5×10^{-4} M) was added. Five microliters from this mixture was placed on an Al foil covered microscope slide and investigated by SERS in liquid drop form. For each sample, 3 measurements were averaged. An integration time of 40 s was used for each spectrum, the laser power on the sample being approximately 50 mW.

All SERS spectra from the peripheral blood genomic DNA were acquired using the same batch of nanoparticles in order to prevent any bias because of differences between the SERS substrate.

Spectra processing and statistical analysis The SERS spectra were preprocessed by linear background subtraction and unit area normalization, using The Unscrambler (Camo Software, 10.1). The subsequent statistical analysis was performed in MATLAB (Mathworks), using the Statistics and Machine Learning toolbox.

For the univariate statistical analysis, we took the normalized intensity of the 1005 cm^{-1} band assigned to the methyl rocking vibration band of 5-methylcytosine, for which we calculated the receiver operating characteristic (ROC) curve. The cutoff point was calculated based on Youden's J statistics.

For analyzing the SERS spectra from genomic DNA, we employed support vector machine (SVM) and principal component analysis-linear discriminant analysis (PCA-LDA). For the PCA-LDA, we used the first 7 PCs as input, which were inspected visually and contain meaningful SERS bands. Thus, the discrimination of the two groups is made strictly based on the SERS signal contained in the 7 PCs. For each test, we used a 5-fold per patient cross-validation. Thus, 80% of the spectra were used to train the classification model while the remaining spectra were used for validation. The 5-fold partition was repeated 1000 times.

Results

In this study, the SERS substrate was represented by colloidal hya-AgNPs, which contain a concentration of 2.4 mM Cl^- released from the synthesis process. The hya-AgNPs were SERS activated with Ca^{2+} 5×10^{-4} M, since in our previous studies we showed that when ions such as Ca^{2+} and Cl^- adsorb onto the metal nanoparticle surface, they generate specific SERS active sites [5, 6]. In particular, the activation of the metal surface with Ca^{2+} ions promotes the chemisorption of the Cl^- onto the metal surface. Further, Cl^- mediates the chemisorption of DNA onto the metal surface, thus playing a crucial role in recording the SERS spectra of DNA [5, 6]. The role of Cl^- ions can be understood by noticing that the SERS spectra of genomic DNA are dominated by bands specific to positively charged nucleotides [8]. Specifically, the bands at 730 and 1330 cm^{-1} can be assigned to adenine nucleotides, while the SERS bands at 680 and 1005 cm^{-1} are assigned to cytosine. Therefore, since Cl^- ions are negatively charged, they facilitate the chemisorption of positive analytes [5, 9].

Table 1 The concentration and purity of the DNA samples from acute myelogenous leukemia (AML) patients and control (CTRL) subjects

	Nr. of samples	Average DNA concentration (range) ng/μl	Average 260/280 ratio (range)
AML	17	43.20 (4.25–91)	1.78 (0.88–2.05)
CTRL	17	47.73 (21.5–132)	1.82 (1.74–193)

In order to show that the cancer-specific cytosine methylation pattern of DNA can be evidenced by SERS, we first compared the SERS spectra of cytosine and 5-methylcytosine (Fig. 1).

The comparative SERS spectrum of cytosine and 5-methylcytosine shows that the latter displays a distinct band at 1002 cm^{-1}, attributed to the rocking vibration of -CH_3 group in methylated cytosine nucleotides [10]. The band is absent from the SERS spectrum of cytosine. Thus, the 1002 cm^{-1} band represents a characteristic spectral pattern of methylated cytosine.

In order to highlight the spectral differences between cancer and normal genomic DNA in regard to the methylation status, we recorded the SERS spectra of genomic DNA samples extracted from a normal cell line (HaCaT) and an AML cell line (THP-1), as shown in Fig. 2.

Figure 2 presents characteristic SERS spectra of normal DNA and cancer DNA, showing that the SERS intensity of the 1005 cm^{-1} band attributed to the rocking vibration of -CH_3 group in methylated cytosine nucleotides has a weaker intensity in the case of cancer DNA, compared to normal DNA. This result is in accordance with the well-known global hypomethylation of cancer DNA which occurs during the malignant transformation of the cells [1].

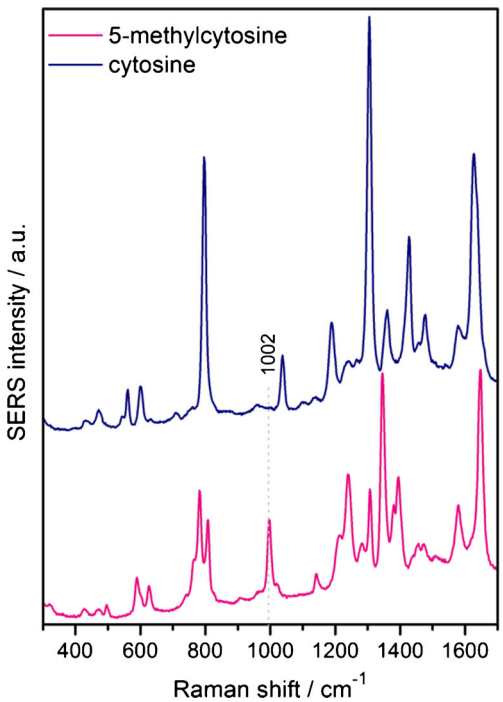

Fig. 1 SERS spectra of cytosine and 5-methylcytosine (100 ng/μl) acquired with hya-AgNPs (532 nm excitation wavelength, 10 s integration time, and 20 mW laser power). The rocking vibration of -CH_3 group of 5-methylcytosine at 1002 cm^{-1} is highlighted as a distinctive SERS band, which is missing in the case of unmethylated cytosine [10]

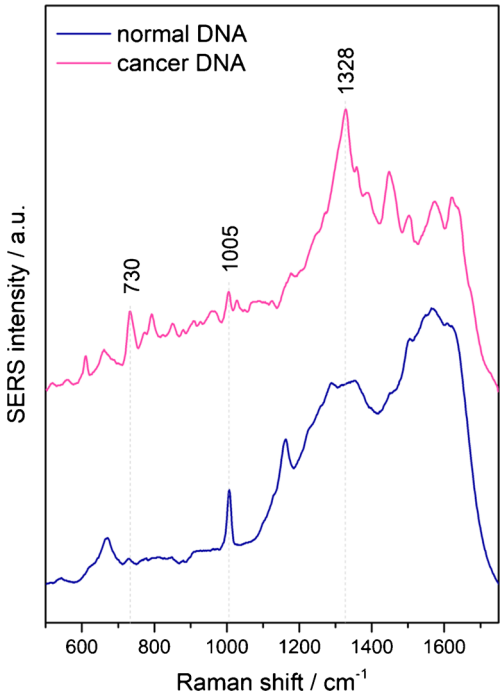

Fig. 2 SERS spectra of normal DNA and cancer DNA (10 ng/μl) extracted from cultured cell lines. The SERS bands attributed to 5-methylcytosine (1005 cm^{-1}) and adenine (730 and 1328 cm^{-1}) are highlighted

Moreover, another distinctive spectral feature, highlighted in Fig. 2, is represented by the different intensities of the SERS bands at 730 and 1328 cm^{-1} attributed to adenine [11]. Thus, the SERS intensities of the bands at 730 and 1328 cm^{-1} were higher in the case of cancer DNA compared to normal DNA. The differences in the SERS intensities of the adenine bands stem also from the cancer-specific DNA methylation pattern, which influences the adsorption geometry onto the metal surface. These results are in line with findings reported by Sin et al., which demonstrated that the methylation landscape of cancer DNA drives the preferential self-assembly of cancer DNA onto metal nanoparticles [4].

Next, we analyzed by SERS peripheral blood genomic DNA from n = 17 patients with acute myeloid leukemia that were in hematological remission and n = 17 healthy volunteers, with the aim of highlighting the spectral differences between the two groups. The marker SERS bands attributed to 5-methylcytosine (1005 cm^{-1}) and adenine (730 and 1328 cm^{-1}) are clearly evident (Fig. 3a) and the differences in the SERS intensity of these bands between the AML and control groups perfectly mirror the behavior seen in the case of the DNA extracted from cultured cell lines (Fig. 2).

Thus, the SERS spectra of the DNA from AML patients display a lower intensity of the 5-methylcytosine band at 1005 cm^{-1} and a higher intensity for the adenine bands at 730 and 1328 cm^{-1} compared to controls (Fig. 3a). The individual SERS spectra of DNA extracted from peripheral blood are presented in ESM (Fig. S2).

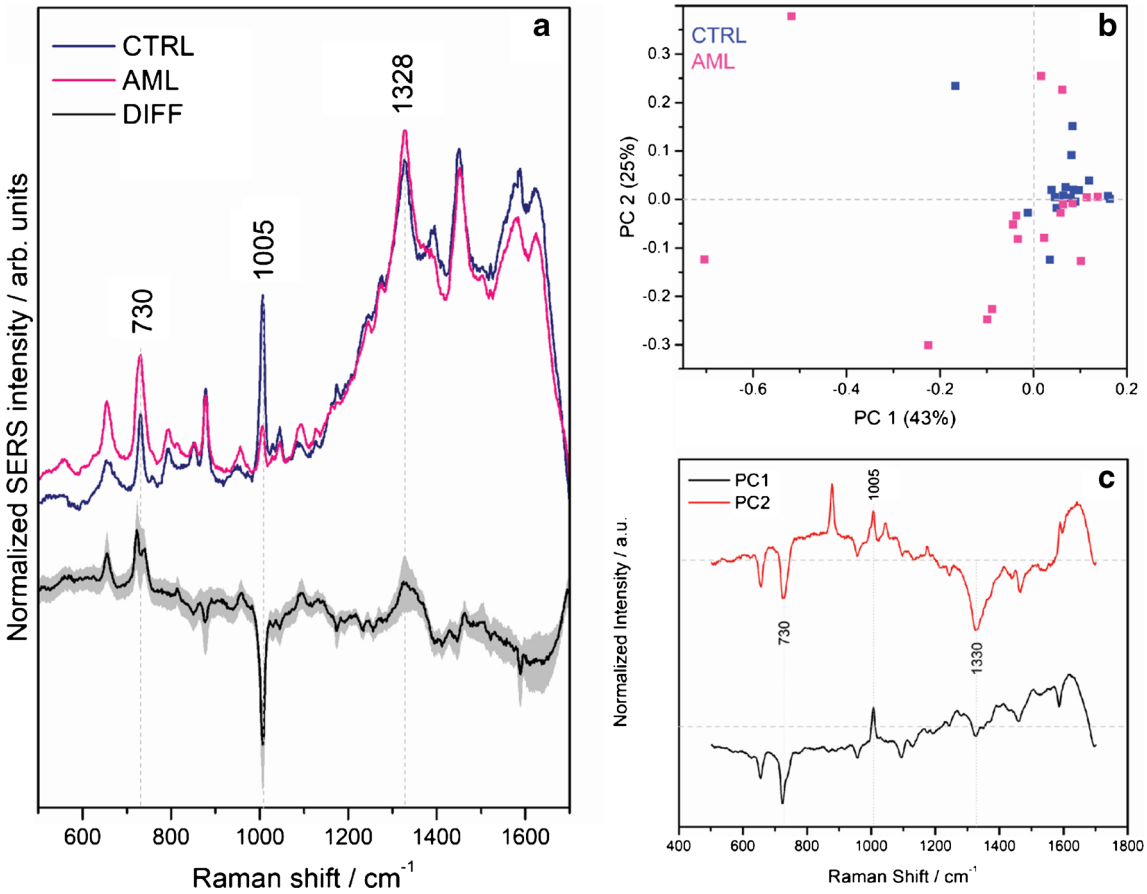

Fig. 3 **a** The average SERS spectra of peripheral blood genomic DNA from the acute myeloid leukemia (AML) group, control (CTRL) group, and the difference spectrum (DIFF). The spectra were preprocessed by linear baseline subtraction and unit area normalization. The shaded area represents the standard deviation. **b** The score plot for the first 2 PCs yielded by principal component analysis (PCA). **c** Loadings plots of the first 2 PCs yielded by PCA. The loadings plots confirm the negative correlation between the 1005 cm^{-1} bands (positive) and the 1330 and 733 cm^{-1} SERS bands (negative)

To better visualize the spectral differences between the SERS signal of the AML and control groups, we performed PCA, which is a well-known multivariate statistical analysis method used for dimensionality reduction and for highlighting the SERS bands that feature a significant variation across the data set. The score plot and loadings spectra are presented Fig. 3b and c and show the negative correlation between the 1005 cm^{-1} band of 5-methylcytosine and the adenine bands at 730 and 1328 cm^{-1} SERS bands.

Based on their SERS spectral shape, the DNA samples extracted from AML patients could be discriminated from control samples using both, univariate and multivariate data analysis techniques. In the case of the univariate analysis, we used the normalized intensity of the 1005 cm^{-1} band, which yielded an area under the curve (AUC) of 0.837 (Fig. 4).

Based on Youden's index, the optimal cutoff value for the normalized intensity of the 1005 cm^{-1} band was 0.028, which corresponded to a sensitivity of 82.35%, a specificity of

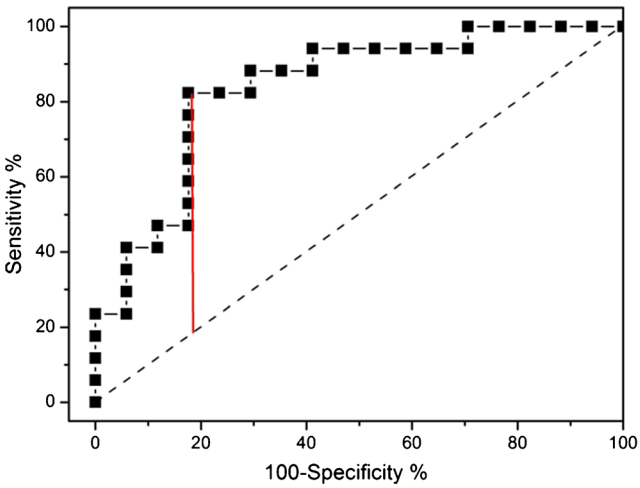

Fig. 4 The receiver operating characteristic (ROC) curve corresponding to the normalized SERS intensity of the 1005 cm^{-1} band attributed to 5-methylcytosine. The area under the curve is 0.837. The optimal cutoff value was chosen based on Youden's index (red line)

82.35%, and an overall accuracy of 82.35% (red line in Fig. 4). The Spearman rank correlation coefficient between the concentration of DNA samples and the normalized intensity of 1005 cm^{-1} was 0.038, which excludes the DNA concentration as a confounding factor for the discrimination between cancer and normal DNA.

The classification accuracy yielded by univariate analysis (82.35%) was similar to that obtained by PCA-LDA and SVM multivariate analysis (Table 2).

PCA-LDA and SVM yielded an overall accuracy of 82.2% and 75.3%, respectively. The sensibility yielded by PCA-LDA and SVM was 75% and 82%, respectively, while the specificity was 89% and 68%, respectively.

Discussion

This study demonstrates that SERS spectroscopy is able to sense the unique methylation pattern of cancer DNA, a strategy which could be used for the diagnosis and follow-up of AML patients. The results obtained on real-life peripheral blood genomic DNA samples showed that AML patients and controls can be discriminated with a sensitivity of 82%, specificity of 82%, and an overall accuracy of 82%. This result is remarkable, since it suggests that cancer DNA can be detected even in the milieu of normal DNA, as it is the case with the DNA extracted from the peripheral blood of AML patients in hematological remission. The similitude between sensitivity and specificity also suggests that the probability of AML patients to display abnormal DNA methylation levels is similar to the probability of control subjects to display normal DNA methylation levels. This result is in line with the study reported by Sin et al., which demonstrated that the methylation landscape of cancer DNA drives the preferential self-assembly of cancer DNA onto metal nanoparticles compared to normal DNA [4].

The here presented data is of great potential use in the clinic for AML patients that undergo allogeneic stem cell transplantation. Currently, the tumor burden of leukemia patients is monitored through NGS or flow cytometry [12, 13]. However, the enormous genomic and phenotypic complexity of AML makes monitoring particularly challenging [14]. Thus, should the SERS-based assay be validated, it would provide clinicians a valuable tool for the follow-up of AML patients that undergo allogeneic stem cell transplantation. The detection of circulating cancer DNA based on its specific methylation pattern was recently demonstrated using immunoprecipitation with antibodies against 5-methylcytosine, followed by NGS of circulating tumor DNA [3]. However, the detection of cancer DNA by NGS requires the amplification of the DNA, which limits its use in the clinical and research settings.

As opposed to the majority of previous SERS studies performed on DNA, which have focused on short, chemically synthesized single-stranded and double-stranded DNA [15, 16], our study on genomic DNA samples successfully identified linked the band at 1005 cm^{-1} with the methylation status of cytosine residues, one of the most well understood form of epigenetic regulation. Epigenetic modifications, including cytosine methylation, are also absent in the case of complementary DNA [17], which results from RNA by reverse transcription. Besides the SERS band at 1005 cm^{-1}, epigenetic modifications also perturb the adsorption of DNA onto metal surfaces DNA [4], which translates into modified spectral shapes, such as the increase in the adenine bands described in this study. Thus, our study raises awareness regarding the importance of cytosine methylation and possibly other epigenetic modifications when analyzing DNA by SERS.

Although SERS analysis of genomic DNA from cancer cell lines has been reported before [16], the analysis of genomic DNA from AML patients is much more difficult since it contains both malignant DNA and non-malignant DNA from normal hematopoiesis. One study by Trau et al. described direct SERS detection of complementary DNA from prostate cancer patients [17]. However, complementary DNA differs from genomic DNA since it lacks any epigenetic modification. Moreover, the authors amplified their samples prior to SERS analysis using multiplex reverse transcription-recombinase polymerase amplification (RT-RPA), whereas our protocol did not involve any DNA amplification step.

Our study represents one of the first insights regarding the analysis of the epigenetic modifications by SERS in the case of real-life genomic DNA samples, opening new avenues for the diagnosis and follow-up of cancer patients.

Table 2 The confusion matrix corresponding to the principal component analysis-linear discriminant analysis (PCA-LDA) and support vector machine (SVM) models in the case of the SERS spectra of DNA from acute myeloid leukemia (AML) patients and control subjects (control). The analysis was performed using a 5-fold cross-validation scheme repeated 1000 times

		PCA-LDA		SVM	
		Predicted			
		AML (%)	Control (%)	AML (%)	Control (%)
Reference	AML	75.3	24.7	82.4	17.6
	Control	10.4	89.6	31.8	68.2

Conclusions

In this preliminary study, we demonstrated the use of SERS for detecting cancer DNA based on its characteristic methylation pattern, without prior amplification of DNA. Similar distinctive SERS spectral features in the intensity of 5-

methylcytosine (1005 cm^{-1}) and adenine (730 and 1328 cm^{-1}) marker bands of the AML and control groups were observed in the case of DNA extracted from cultured cell lines and peripheral blood genomic DNA. Thus, cancer DNA presented a decreased intensity of the 1005 cm^{-1} band of 5-methylcytosine compared to normal DNA, in line with the well-described hypomethylation of cancer DNA, whereas the SERS intensities of the adenine bands at 730 and 1328 cm^{-1} were higher in the case of cancer DNA compared to normal DNA. The differences in the SERS intensities of the adenine bands stem also from the cancer-specific DNA methylation pattern, which influences the DNA adsorption geometry onto the metal surface. Blood genomic DNA from AML patients was discriminated from that of controls with an overall accuracy of 82.35% (AUC = 0.837), based on the 1005 cm^{-1} band of methylated cytosine. PCA-LDA and SVM yielded an overall classification accuracy of 82.2% and 75.3%, respectively. The label-free SERS detection of cancer DNA without requiring any DNA amplification step represents a promising strategy that could be translated in the clinical setting for the screening and follow-up of cancer patients.

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Compliance with ethical standards All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Ehrlich M. DNA hypomethylation in cancer cells. Epigenomics. 2009;1(2):239–59.
2. Kelly AD, Kroeger H, Yamazaki J, Taby R, Neumann F, Yu S, et al. A CpG island methylator phenotype in acute myeloid leukemia independent of IDH mutations and associated with a favorable outcome. Leukemia. 2017;31(10):2011–9.
3. Shen SY, Singhania R, Fehringer G, Chakravarthy A, Roehrl MHA, Chadwick D, et al. Sensitive tumour detection and classification using plasma cell-free DNA methylomes. Nature. 2018;563(7732):579–83.
4. Sina AA, Carrascosa LG, Liang Z, Grewal YS, Wardiana A, Shiddiky MJA, et al. Epigenetically reprogrammed methylation landscape drives the DNA self-assembly and serves as a universal cancer biomarker. Nat Commun. 2018;9(1):4915.
5. Leopold N, Stefancu A, Herman K, Todor IS, Iancu SD, Moisoiu V, et al. The role of adatoms in chloride-activated colloidal silver nanoparticles for surface-enhanced Raman scattering enhancement. Beilstein J Nanotechnol. 2018;9:2236–47.
6. Stefancu A, Moisoiu V, Bocsa C, Bálint Z, Cosma DT, Veresiu IA, et al. SERS-based quantification of albuminuria in the normal-to-mildly increased range. Analyst. 2018;143(22):5372–9.
7. Leopold N, Lendl B. A new method for fast preparation of highly surface-enhanced Raman scattering (SERS) active silver colloids at room temperature by reduction of silver nitrate with hydroxylamine hydrochloride. J Phys Chem B. 2003;107(24):5723–7.
8. Srinivasan AR, Sauers RR, Fenley MO, Boschitsch AH, Matsumoto A, Colasanti AV, et al. Properties of the nucleic-acid bases in free and Watson-Crick hydrogen-bonded states: computational insights into the sequence-dependent features of double-helical DNA. Biophys Rev. 2009;1(1):13–20.
9. Doering WE, Nie S. Single-molecule and single-nanoparticle SERS: examining the roles of surface active sites and chemical enhancement. J Phys Chem B. 2002;106(2):311–7.
10. Śanchez-Cortés S, Garcia-Ramos JV. SERS of cytosine and its methylated derivatives on metal colloids. J Raman Spectrosc. 1992;23(1):61–6.
11. Xu L-J, Lei Z-C, Li J, Zong C, Yang CJ, Ren B. Label-free surface-enhanced raman spectroscopy detection of DNA with single-base sensitivity. J Am Chem Soc. 2015;137(15):5149–54.
12. Orsmark-Pietras C, Lilljebjörn H, Rissler M, Lazarevic V, Arvanitakis A, Ehinger M, et al. Comprehensive prospective next generation sequencing of acute myeloid leukemia. Blood. 2015;126(23):3830.
13. Chen X, Cherian S. Acute myeloid leukemia immunophenotyping by flow cytometric analysis. Clin Lab Med. 2017;37(4):753–69.
14. Ley TJ, Miller C, Ding L, Raphael BJ, Mungall AJ, Robertson A, et al. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med. 2013;368(22):2059–74.
15. Garcia-Rico E, Alvarez-Puebla RA, Guerrini L. Direct surface-enhanced Raman scattering (SERS) spectroscopy of nucleic acids: from fundamental studies to real-life applications. Chem Soc Rev. 2018;47(13):4909–23.
16. Panikkanvalappil SR, Mahmoud MA, Mackey MA, El-Sayed MA. Surface-enhanced Raman spectroscopy for real-time monitoring of reactive oxygen species-induced DNA damage and its prevention by platinum nanoparticles. ACS Nano. 2013;7(9):7524–33.
17. Wang J, Koo KM, Wee EJH, Wang Y, Trau M. A nanoplasmonic label-free surface-enhanced Raman scattering strategy for non-invasive cancer genetic subtyping in patient samples. Nanoscale. 2017;9(10):3496–503.