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Non-invasive detection of hepatocellular carcinoma serum metabolic profile through surface-enhanced Raman spectroscopy

Rui Xiao, PhD[a,1], Xuhui Zhang, PhD[b,1], Zhen Rong, PhD[a], Bingshui Xiu, PhD[b], Xiqin Yang, MSc[b], Chongwen Wang, MSc[a], Wende Hao, MMedSc[c], Qi Zhang, MSc[d], Zhiqiang Liu, PhD[b], Cuimi Duan[b], Kai Zhao, MMedSc[e], Xu Guo, MMedSc[f], Yawen Fan, MSc[b], Yanfeng Zhao, MMedSc[b], Heather Johnson, PhD[g], Yan Huang, MD[c], Xiaoyan Feng, PhD[b,*], Xiaohong Xu, MMedSc[h,*], Heqiu Zhang, MMedSc[b,*], Shengqi Wang, PhD[a,*]

[a]*Beijing Institute of Radiation Medicine, Beijing, China*
[b]*Department of Bio-diagnosis, Institute of Basic Medical Sciences, Beijing, China*
[c]*Affiliated 307 Hospital, Beijing, China*
[d]*National Center of Biomedical Analysis, Beijing, China*
[e]*Department of Radiotherapy and Chemotherapy, Tangshan City People's Hospital, Tangshan, China*
[f]*Clinical Laboratory, 252 Hospital of PLA, Baoding, China*
[g]*Olympia Diagnostics, Inc. Sunnyvale, CA, USA*
[h]*Zhejiang Cancer Hospital, Hangzhou, China*

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Abstract

The present study aims to identify distinctive Raman spectrum metabolic peaks to predict hepatocellular carcinoma (HCC). We performed a label-free, non-invasive surface-enhanced Raman spectroscopy (SERS) test on 230 serum samples including 47 HCC, 60 normal controls (NC), 68 breast cancer (BC) and 55 lung cancer (LC) by mixing Au@AgNRs with serum directly. Based on the observed SERS spectra, discriminative metabolites including tryptophan, phenylalanine, and etc. were found in HCC, when compared with BC, LC, and NC ($P < 0.05$ in all). Common metabolites-proline, valine, adenine and thymine were found in HCC, BC and LC with compared to NC group ($P < 0.05$). Importantly, Raman spectra of HCC serum biomarker AFP were firstly detected to analyze the HCC prominent peak. Orthogonal partial least squares discriminant analysis was adopted to assess the diagnostic accuracy; area under curve value of HCC is 0.991. This study provides new insights into the HCC metabolites detection through Raman spectroscopy.

Key words: Surface-enhanced Raman scattering; Hepatocellular carcinoma; Au@Ag nanorods; Serum metabolites; Cancer detection

Cancer is a major public health problem worldwide. A total of 14 million new cases and 8.2 million cancer-related deaths were reported in 2012 (WHO). Early and accurate detection of cancer cannot only reduce mortality and morbidity but also can improve the quality of life of patients. Raman spectroscopy measures the inelastic scattering process and presents fingerprinting type information on the molecular composition and structure of a sample. It has emerged as a powerful technique for the diagnosis of biological samples or disease states. However, the Raman signals from biological samples are usually weak. This disadvantage limits the clinical applications of this technology. SERS demonstrates as much as 10^{13} to 10^{15} times of Raman signal enhancement through the adsorption of sample molecules onto metal nanostructures. The use of SERS has increased exponentially in the past three decades. SERS has been widely applied in the analysis of various biological samples, such as proteins, nucleic acids, living or fixed cells, and excised tissues.

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*Corresponding author.
E-mail addresses: xyfeng2002@126.com (X. Feng), zjhzxxh@163.com (X. Xu), zhangheqiu2004@126.com (H. Zhang), sqwang@bmi.ac.cn (S. Wang).
[1] These authors contributed equally to this work

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In recent years, SERS technologies have been extensively utilized for in vitro cancer detection and diagnostics.[1-3] The application of SERS technique in cancer diagnosis and evaluation in a number of human biological samples, especially blood samples, has been investigated.[3,4] Major progress has also been made in label-free SERS technologies in the diagnosis and prediction of cancer with patients' plasma or serum samples in cervical, bladder, gastric, nasopharyngeal, prostate, and breast cancers.[5-10] Blood plasma or serum is a metabolite pool derived from nearly all the organs and tissues of the human body and can be considered a window of the overall metabolic changes in an organism. Raman spectroscopy is one of the rapidly developing analytical technologies that have made the most important steps in the workflow of metabolomic research.[11,12] It relies on the vibrational frequencies of metabolites to provide a fingerprint of metabolism. Although selectivity and sensitivity in Raman spectroscopy are not as high as those in mass spectrometry, the former has the advantages of minimal sample preparation, low cost, and rapidity.

HCC is the 5th most common type of cancer worldwide and the 3rd most common reason for cancer-related deaths.[13] HCC represents more than 90% of liver cancer and is usually diagnosed at late and advanced stages. The most commonly used serum marker in the clinical diagnosis of HCC is alpha-fetoprotein (AFP). Almost one-third of early-stage HCC patients exhibit the same level of AFP as healthy subjects.[14,15] Thus, AFP has limited sensitivity and specificity for the diagnosis of early-stage HCC. Ultrasonography is commonly used in HCC screening because of its cost effectiveness and non-invasiveness. However, it is not sensitive enough to detect liver tumors in patients with a cirrhotic background.[16] Thus, a reliable diagnostic method that can be used for early and accurate detection of HCC must be established.

Cancer cells produce specific metabolites, which can be detected by specific analytical techniques. Hepatic diseases are topics of great concern in metabonomic studies.[17,18] Although serum Raman spectroscopy has exhibited its potential in the diagnosis of HCC,[19] the performance of serum SERS in the detection of metabolic profiles in HCC patients remains to be addressed. Moreover, characterizing the general serum metabolic alterations of different types of cancer is still necessary.

In the present study, an improved SERS technique was utilized as a sensitive detection method for cancer-related serum metabolism in HCC. LC, BC, and normal groups were formed for comparison. To our knowledge, this paper is the first to report on the detection of serum metabolic profiles in HCC patients through SERS. This study is also the first to detect and compare serum SERS in three types of cancer to determine the general serum metabolic alterations among cancer patients. In addition, Raman spectra of HCC serum biomarker AFP were detected to analyze the HCC prominent peak for the first time.

Methods

Synthesis of AuNRs and Au@Ag NRs

Au@Ag nanorods (Au NRs) were synthesized according to the seeding growth method reported by Nikoobakht et al.[20] First, the seed solution was prepared by mixing 5 mL of 0.1 M CTAB solution with 42 μL of 29 mM $HAuCl_4$. Then, 0.3 mL of 10 mM $NaBH_4$ was added with vigorous stirring for 10 min. Second, the resulting seed solution was utilized in the preparation of Au NRs. Briefly, 0.4 mL of 10 mM $AgNO_3$ was added to 40 mL of 0.1 M CTAB solution. Then, 0.8 mL of 29 mM $HAuCl_4$ was added and mixed. To this solution, 0.32 mL of 0.1 M ascorbic acid was added with gentle mixing. Finally, 120 μL of seed solution was added. The entire solution was kept at 30 °C overnight without any further stirring. A total of 24 mL of the as-prepared Au NRs was centrifuged twice at 8000 rpm for 10 min to remove the excess reagents. The precipitate was re-dispersed in 12 mL of deionized water. This purified GNR solution was then added to 30 mL of 0.04 M CTAB aqueous solution under vigorous stirring, with the temperature maintained at 30 °C. Then, 1.56 mL of 0.1 M ascorbic acids, 2.2 mL of 10 mM $AgNO_3$, and 3.48 mL of 0.1 M NaOH solutions were added sequentially. The color of the solution changed rapidly from brown to dark green, indicating the formation of Au@Ag NRs.

ERS measurements

A 5 μL sample solution was mixed with 5 μL of prepared Au@Ag NRs. The mixture was then kept undisturbed at 4 °C for 3 h. A droplet (2.5 μL) of the sample suspension was added to a Si wafer and then dried at room temperature. Raman spectra were obtained with a portable Raman system B&W Teki-Raman Plus BWS465-785H spectrometer equipped with a back-illuminated CCD detector cooled at −2 °C. Samples were excited with a 785 nm laser with a power of 50 mW. SERS spectra were obtained with a total acquisition of 20s for each SERS spectrum, and the spectrum was measured over a wave number range of 600 cm^{-1} to 1750 cm^{-1}, with a 3.5 cm^{-1} spectral resolution. The spectrum of each sample was obtained from the average of three spectra measured at random spots.

Patients and collection of serum samples

All subjects were properly informed, and written informed consent was obtained from each individual. All methods were carried out in accordance with the approved guidelines of the Ethics Committee of the Institute of Basic Medical Sciences and Affiliated 307 Hospital. All experimental protocols were approved by the Ethics Committee of the Institute of Basic Medical Sciences and Affiliated 307 Hospital, including any relevant details. A total of 230 human fasting serum specimens were utilized in this study. After overnight fasting, a single 4 mL peripheral blood samples were obtained from the study subjects and collected in serum separator tubes. Serum separator tubes were centrifuged for 15 minutes at 2000 RPM within one hour of collection. Then, blood serum samples from healthy subjects and patients were obtained and, then aliquoted and stored at −80 °C until the time of analysis. A total of 30 male and 30 female healthy control samples, 47 HCC serum samples, 68 BC serum samples, and 55 LC serum samples were obtained from Affiliated 307 Hospital. Patients with HCC, LC, and BC were diagnosed by a clear clinical laboratory, imaging evidence, or histopathologically diagnosed after tumor excision. Tumor stages were classified based on the staging system of the American Joint Committee on Cancer.