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Age-specific discrimination of blood plasma samples of healthy and ovarian cancer prone mice using laser-induced breakdown spectroscopy☆

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ABSTRACT

Epithelial ovarian cancer (EOC) mortality rates are strongly correlated with the stage at which it is diagnosed. Detection of EOC prior to its dissemination from the site of origin is known to significantly improve the patient outcome. However, there are currently no effective methods for early detection of the most common and lethal subtype of EOC. We sought to determine whether laser-induced breakdown spectroscopy (LIBS) and classification techniques such as linear discriminant analysis (LDA) and random forest (RF) could classify and differentiate blood plasma specimens from transgenic mice with ovarian carcinoma and wild type control mice. Herein we report results using this approach to distinguish blood plasma samples obtained from serially bled (at 8, 12, and 16 weeks) tumor-bearing TgMISIIR-TAg transgenic and wild type cancer-free littermate control mice. We have calculated the age-specific accuracy of classification using 18,000 laser-induced breakdown spectra of the blood plasma samples from tumor-bearing mice and wild type controls. When the analysis is performed in the spectral range 250 nm to 680 nm using LDA, these are 76.7 (±2.6)%, 71.2 (±1.3)%, and 73.1 (±1.4)%, for the 8, 12 and 16 weeks. When the RF classifier is used, we obtain values of 78.5 (±2.3)%, 76.9 (±2.1)% and 75.4 (±2.0)% in the spectral range of 250 nm to 680 nm, and 81.0 (±1.8)%, 80.4 (±2.1)% and 79.6 (±3.5)% in 220 nm to 850 nm. In addition, we report, the positive and negative predictive values of the classification of the two classes of blood plasma samples. The approach used in this study is rapid, requires only 5 μL of blood plasma, and is based on the use of unsupervised and widely accepted multivariate analysis algorithms. These findings suggest that LIBS and multivariate analysis may be a novel approach for detecting EOC.

I. Introduction

Despite intensive research efforts, cancer remains a lethal disease that affects millions of people around the world. In 2014, it accounted for the deaths of nearly 600,000 Americans, or 1600 people per day [1,2]. Sadly, despite many efforts and progress in cancer diagnosis and treatment, the human cost of this disease remains very high. A key challenge is to be able to detect and diagnose in its early stages of development. Early detection provides medical professionals much better possibilities for interventions to prevent or halt further progression, which would lead to a reduction in mortality and morbidity. Thus, for a large number of cancers, it is essential to develop and implement tests that provide signs of the disease prior to its spread from the site of origin. In addition, such tests would be even more useful if they are minimally invasive and can be made widely available.

Despite rich and diverse research efforts, early detection and diagnosis of epithelial ovarian cancer (EOC) remains a significant challenge [3,4]. In the United States, approximately 22,000 women are diagnosed with EOC each year. This disease is the leading cause of death among gynecological cancers with ~14,000 deaths annually. Worldwide, it is estimated that there are 220,000 new cases of EOC and 140,000 deaths caused by the disease annually. EOC typically develops in the absence of specific symptoms; therefore, the majority (>70%) of cases are discovered when the disease is already at an advanced stage [5,6]. The 5-year survival rate for patients diagnosed with advanced (stage III and IV) cancers is 30–40%. However, if EOC is diagnosed at an early stage, this rate ranges from 60% to 90%, depending on the degree of tumor differentiation [7,8]. These statistics demonstrate that successful early detection and diagnosis of EOC would be particularly useful for improving prognosis and decreasing the mortality rate.

One of the important and promising strategies for earlier cancer diagnosis includes identification and detection of cancer-associated

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biomarkers through non- or minimally-invasive approaches. Such biomarkers would be ideally produced either by the tumor itself or by the surrounding tissues and can be found in a variety of biological fluids. Also important is the ability to yield specific and sensitive signatures accurate enough for the early detection of the disease. Changes in biomarkers present in biological fluids are particularly attractive because they can reveal the state of a cell. Cancer Antigen 125 (CA-125) is a protein widely used as a biomarker for detection of recurrent EOC [9,10]. Recently, researchers reported the simultaneous detection of multiple candidate biomarkers for the early detection of EOC instead of a single one leads to increased specificity and sensitivity for cancer detection [11,12] and the use of multicolor quantum dots and metal nanoparticles for tagging biomarkers for diagnostics of cancer [13,14]. Recently, we reported on a technique, Tag-laser induced breakdown spectroscopy (Tag-LIBS), which relies on tagging CA-125 with metal microparticles in blood serum prior to performing LIBS measurements [15–17]. We have shown that this approach provides an estimated near single molecule per particle efficiency of CA-125 in human blood plasma and can, in principle, be generalized for the sensitive and simultaneous detection of multiple biomarkers in biomedical fluids and/or tissues.

Another approach that has attracted much attention is the use of classification techniques on spectroscopic data of biological samples acquired using methods such as surface-enhanced laser desorption and ionization (SELDI) [10], matrix-assisted laser desorption/ionization (MALDI) [18] and at the genomic level, DNA chip technology [19,20].

LIBS has been used by a number of researchers to classify various samples [see for example, 22]. A number of review papers have been devoted to the theoretical, experimental and applications of the technique [23–26]. In this paper, we report on the first use of laser–induced breakdown spectroscopy (LIBS) for the blind classification of blood samples extracted from wild type and transgenic mice with ovarian tumors. The blood samples used for this study were collected from transgenic TgMISIIR-TAg mice in which transgene positive females develop bilateral epithelial ovarian cancer with metastatic spread to peritoneal organs and the formation of ascites [27]. The blood samples collected from wild type (transgene negative) female littermates were used as controls. Unlike Tag-LIBS, this approach does not require a specific sample preparation prior to performing LIBS measurements and classification.

II. Transgenic mice, sample preparation and experimental set-up

The C57BL/6 TgMISIIR-TAg transgenic mice used for this study have been described [8,20,28,29]. Female C57BL/6 TgMISIIR-TAg transgenic mice develop bilateral ovarian tumors with 100% penetrance [28,29]. Tumors develop with variable latency and female TgMISIIR-TAg transgenic mice survive an average of 152 days [28,30]. The size of ovaries remains relatively normal (<10 mm^3) for the first 70–85 days of life, and undergoes a substantial increase in growth between 100 and 150 days of age, reaching a volume of about 50 mm^3 in a median time of 110 days. Although ovaries appear ostensibly normal in structure and size in young mice, postmortem examination of the ovaries shows the presence of small numbers of TAg+ tumor cells as early as 28 days of age [28,29]. Although the size of the ovaries remains normal for the first 2–3 months, the early presence of tumor cells in one-month old mice suggests that potential cancer signatures may exist in the blood samples of young transgenic mice. For this reason, we collected blood from mice at 8, 12 and 16 weeks of age.

All of the blood specimens used in this study were collected from mice bred at Fox Chase Cancer Center under an Institutional Animal Care and Use Committee (IACUC) approved protocol. Male C57BL/6 TgMISIIR-TAg transgenic mice were bred with wild type C57BL/6 female mice to produce female C57BL/6 TgMISIIR-TAg transgenic and wild type littermate control mice. Prior to blood collection, mice were anesthetized with 2–3% isoflurane in O_2. Blood was successively collected from each mouse at 8, 12 and 16-weeks of age. Blood was collected from the retro-orbital sinus (three draws/mouse, alternating eyes) using heparinized Natelson blood collecting tubes and following collection, the blood collection tubes were centrifuged to compact cells. The tubes were cut just above the cellular component and the plasma is transferred to sterile micro-centrifuge tubes. Plasma specimens were stored at −80 °C until all of the specimens were collected at which time they were shipped on dry ice for subsequent LIBS measurement and analysis.

A total of 56 mice (n = 28 each, transgenic and wild type mice) were bred and bled at 8, 12 and 16 weeks of age yielding a total of 168 blood samples. Six specimens contained insufficient material, leaving 162 specimens for analysis in this study: 28 transgenic "cancer" and 26 wild type "non-cancer" blood plasma samples (Table 1). The blood plasma samples were divided into 3 groups according to age of mice at moment of blood collection: groups 1, 2 and 3 consisted of blood samples from 8, 12 and 16 week-old mice respectively. With the blood plasma

Table 1
List of MISIIRTAg DR26 blood samples.

	ID	Tag	4 weeks	8 weeks	12 weeks
1	K2821	−	N/A	N/A	401
2	K2869	−	N/A	N/A	402
3	K2981	+	203	303	403
4	K2982	−	204	304	404
5	K2987	+	205	305	405
6	K2988	−	206	306	406
7	K2989	+	207	307	407
8	K2995	+	208	308	408
9	K2996	−	209	309	409
10	K3002	+	210	310	410
11	K3008	−	211	311	411
12	K3010	+	212	312	412
13	K3011	+	213	313	413
14	K3016	−	214	314	414
15	K3066	+	215	315	415
16	K3067	−	216	316	416
17	K3068	−	217	317	417
18	K3069	−	218	318	418
19	K3252	−	219	319	419
20	K3253	−	220	320	420
21	K3308	−	221	321	421
22	K3309	+	222	322	422
23	K3310	−	223	323	423
24	K3314	−	224	324	424
25	K3415	−	225	325	425
26	K3416	+	226	326	426
27	K3417	−	227	327	427
28	K3418	+	228	328	428
29	K3419	−	229	329	429
30	K3421	+	230	330	430
31	K3422	+	231	331	431
32	K3423	+	232	332	432
33	K3424	−	233	333	433
34	K3463	+	234	334	434
35	K3464	+	235	335	435
36	K3466	−	236	336	436
37	K3471	+	237	337	437
38	K3472	+	238	338	438
39	K3473	+	239	339	439
40	K3474	−	240	340	440
41	K3475	−	241	341	441
42	K3476	−	242	342	442
43	K3477	−	243	343	443
44	K3478	+	244	344	444
45	K3479	−	245	345	445
46	K3549	−	246	346	446
47	K3550	+	247	347	447
48	K3551	−	248	348	448
49	K3552	+	249	349	449
50	K3553	+	250	350	450
51	K3554	+	251	351	451
52	K3555	+	252	352	452
53	K3556	+	253	353	453
54	K3557	−	254	354	454
55	K3558	+	255	355	N/A
56	K3653	+	256	356	N/A