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Research Letter

Serum lipidomic study reveals potential early biomarkers for predicting response to chemoradiation therapy in advanced rectal cancer: A pilot study

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Abstract

Purpose: Prospective detection of patients with advanced rectal cancer (LARC) who have a higher probability of responding to preoperative chemoradiotherapy (CRT) may provide individualized therapy. Lipidomics is an emerging science dedicated to the characterization of lipid fingerprint involved in different pato-physiological conditions. The purpose of this study is to highlight a typical lipid signature able to predict the tumor response to CRT.

Experimental Design: A prospective global analysis of lipids in 54 sera from 18 LARC patients treated with preoperative CRT was performed. Samples were collected at 3 time points: before (T0), at 14th day and at 28th day of CRT. An open LC-MS/MS analysis was performed to characterize lipid expression at T0. Differential lipids were validated by an independent approach and studied during treatment.

Results: From 65 differential lipids highlighted between responder (RP) vs not responder (NRP) patients, five lipids were validated to predict response at T0: SM(d18:2/18:1), LysoPC (16:0/0:0), LysoPC (15:1(9z)/0:0), Lyso PE (22:5/0:0) and m/z = 842.90 corresponding to a PC containing 2 fatty acids of 40 carbons totally. The levels of these lipids were lower in NRP before treatment. The ROC curve obtained by combining these five lipid signals showed an AUC of 0.95, evidence of good sensitivity and specificity in discriminating groups.

Conflicts of interest: None.

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Conclusion: Our results are in agreement with previous evidences about the role of lipids in determining the tumor response to therapy and suggest that the study of serum lipid could represent a useful tool in prediction of CRT response and in personalizing treatment.

Introduction

Colorectal cancer (CRC) is the third most frequently occurring cancer globally.[1] Preoperative fluoropyrimidine-based chemoradiation therapy (CRT) or short-course radiation therapy followed by total mesorectal excision are the standard treatments for CRC.[2-4] In the effort to personalize treatments, there is increasing interest in predicting which patients will respond to neoadjuvant CRT,[5] especially via investigating easily accessible biological fluids,[6] and in improving response rate and survival outcomes. Several biomarkers have been investigated for their ability to predict outcome in locally advanced rectal cancer (LARC) treated with CRT, but few works have investigated lipids.[7-9] Bioactive lipids are fundamental mediators of a number of biological processes,[10-12] and the implication of lipids in cancer growth and diffusion have already been demonstrated.[13] In this work, we aimed to study serum polar lipids in a prospective cohort of LARC patients before CRT (t0 group), including patients naïve to chemotherapy and radiation therapy. Samples were also collected during CRT (t14 and t28 days), in the effort to correlate the global lipid signature to response to treatment.

Methods

See Appendix E1, available as supplementary material online only at www.practicalradon.org.

Results

Lipidomics biomarker discovery

The serum from 18 patients with LARC (7 women, 11 men)—8 of whom were classified as responders (RPs) and 10 as not responders (NRPs) according to Mandard's tumor regression grading—treated with preoperative CRT was analyzed by liquid chromatography electrospray ionization tandem mass spectometry. Data were converted into a matrix containing m/z signals coupled with

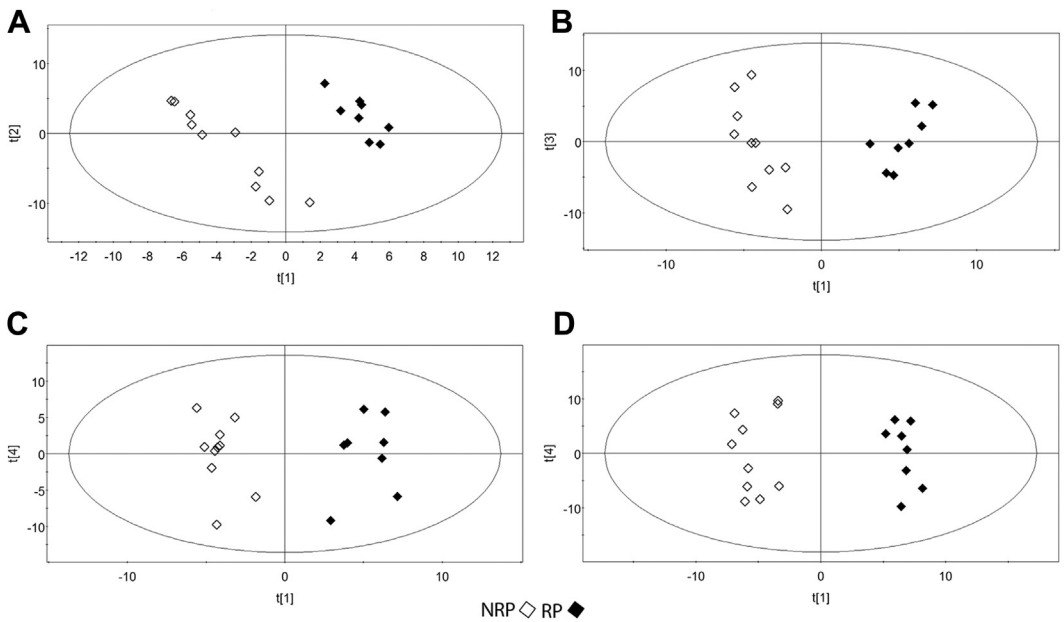

Figure 1 Partial least squares discriminant analysis score plots based on the lipidomics data. Responders (RPs) (represented as full diamonds) and not responders (NRPs; represented as open diamonds) before treatment (t0). The panels show partial least squares discriminant analysis score plots for the analyzed lipids, in particular the phosphatidylcholine/sphingomyelin class (A), phosphatidylethanolamine class (B), phosphatidylglycerol class (C), and phosphatidylserine class (D).

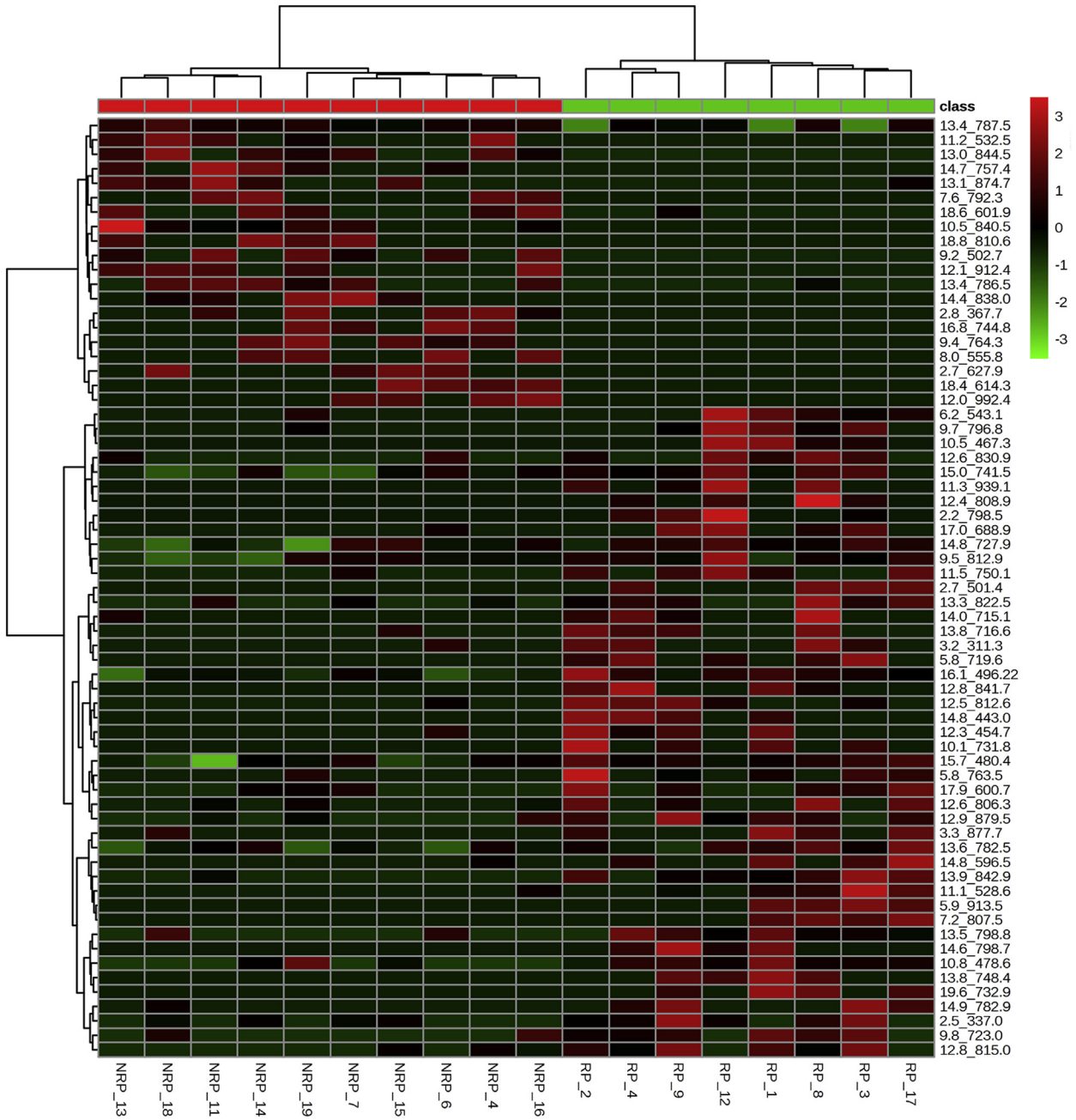

Figure 2 Heat map showing the relative intensity of the 65 differential serum lipids (listed on the right) of each sample (listed at the bottom) before treatment (t0). Samples are divided in 2 groups: RP and NRP. Lipid levels are indicated by a color code: high (red) and low (green). See Fig 1 for abbreviations.

retention time as variables and the patient codes as observations. This dataset was reduced by considering only variables present in at least 50% of patients. Figure 1 shows the lipid classes (including lyso forms) screened. The studied lipids were sphingomyelins (SMs) and phosphatidylcholines (PCs; Fig 1A), phosphatidylethanolamine (Fig 1B), phosphatidylglycerols (Fig 1C), and phosphatidylserines (Fig 1D). Each lipid class screened was reported. In Fig 1A, the score plot of phosphatidylcholine/SM phospholipids is shown, whereas Fig 1B shows the score plot of the phosphatidylethanolamine class; Fig 1C shows the phosphatidylglycerol lipids; and the phosphatidylserine class is reported in Fig1D. The resulting PLS-DA models are reported as score scatter plots in Fig 1, showing clear separation between RP and NRP before treatment. The lipids identified as variable important for the projection (VIP >1) were confirmed through a univariate test. At t0, 65 lipids were identified as significant, with the criteria of VIP >1.5 and $P < .05$ in the univariate test, depicted in Fig 2 as a heat map. The

Table 1 Significant lipids obtained from statistical analysis (VIP >1.5; $P < .05$) in RPs and NRPs at the t0 time point

	RT_m/z	VIP	NRPs		RPs		*t* test value
			Mean	SD	Mean	SD	
PCs/SMs	**14.79_727.86**	**1.86**	**18.03**	**6.28**	**23.84**	**3.91**	**0.037**
	16.14_495.7	**2.59**	**9.88**	**1.53**	**13.49**	**2.42**	**0.001**
	15.72_480.42	**2.27**	**1.46**	**0.62**	**2.24**	**0.39**	**0.008**
	13.44_787.52	2.13	333.95	56.02	183.28	155.44	0.011
	13.51_798.84	1.96	2.21	4.71	8.47	6.02	0.025
	13.86_842.90	**1.71**	**0.08**	**0.25**	**1.88**	**1.26**	**0.0001**
	12.57_830.92	1.70	0.22	0.48	1.01	0.93	0.034
	12.58_806.35	1.87	0.25	0.43	1.53	1.78	0.042
	12.51_812.58	2.21	0.02	0.07	0.29	0.26	0.008
	14.75_757.37	1.83	1.72	2.14	0.00	0.00	0.038
	14.91_782.88	1.93	0.11	0.36	1.53	1.78	0.025
	14.04_715.12	1.89	0.04	0.14	0.40	0.50	0.047
PEs	9.53_812.96	1.92	22.90	15.93	41.24	20.47	0.048
	10.80_478.63	1.78	4.40	8.72	15.31	7.55	0.013
	9.83_723.00	1.78	3.15	6.77	12.80	9.44	0.023
	11.08_528.61	**2.08**	**1.49**	**4.71**	**20.81**	**25.01**	**0.028**
	11.54_750.09	2.46	0.63	2.01	7.78	7.04	0.007
	9.67_796.81	2.23	0.93	2.96	17.07	19.23	0.018
	9.23_502.71	2.39	7.47	7.17	0.00	0.00	0.010
	11.18_532.48	2.05	7.55	9.03	0.00	0.00	0.032
	12.35_454.71	1.96	0.73	2.31	6.06	7.27	0.043
	9.43_764.26	2.15	6.60	7.41	0.00	0.00	0.024
	8.01_555.83	1.92	3.24	4.22	0.00	0.00	0.046
	10.10_731.77	2.21	0.00	0.00	7.10	8.62	0.018
	7.60_792.30	1.91	3.81	4.98	0.00	0.00	0.047
	11.27_939.11	2.16	0.00	0.00	3.70	4.67	0.023
	12.36_808.91	1.98	0.00	0.00	3.24	4.61	0.039
PGs	2.55_337.05	2.11	7.66	10.13	34.40	31.77	0.023
	6.22_543.15	2.07	1.60	5.07	15.31	16.87	0.026
	5.77_763.48	1.86	2.73	8.65	22.98	28.48	0.048
	3.16_311.30	2.01	1.15	3.66	9.54	10.76	0.034
	12.14_912.39	2.14	9.69	10.56	0.00	0.00	0.020
	2.79_367.71	2.04	9.12	10.56	0.00	0.00	0.027
	5.83_719.64	2.54	0.00	0.00	13.05	12.30	0.004
	3.34_877.71	1.92	1.93	6.11	14.62	16.73	0.040
	5.96_913.52	2.33	0.00	0.00	9.74	10.60	0.010
	2.73_627.94	1.70	7.51	9.94	0.00	0.00	0.050
	2.25_798.51	2.02	0.00	0.00	20.04	28.36	0.039
	2.71_501.43	2.34	0.00	0.00	8.52	9.19	0.009
	7.16_807.46	2.32	0.00	0.00	6.22	6.78	0.010
PSs	13.60_782.52	2.35	48.23	37.83	104.02	52.04	0.018
	15.05_741.50	2.44	11.11	9.65	24.79	11.20	0.013
	12.92_879.50	2.45	3.65	7.03	18.01	14.25	0.013
	12.84_815.03	2.49	2.95	5.18	18.46	16.20	0.011
	13.26_822.49	2.43	4.19	7.38	20.42	16.66	0.014
	17.99_600.69	2.13	2.40	3.97	10.04	9.65	0.036
	10.48_840.46	2.08	7.75	9.91	0.00	0.00	0.043
	13.03_844.46	2.78	12.36	10.11	0.00	0.00	0.003
	13.45_786.54	2.50	11.73	10.59	0.71	2.03	0.011
	18.56_601.86	2.11	4.04	4.40	0.39	1.12	0.038
	13.09_874.69	2.04	7.73	8.80	0.76	2.16	0.045
	14.40_838.03	2.04	10.66	13.79	0.00	0.00	0.045
	12.85_841.74	2.12	0.81	2.56	8.56	10.51	0.038
	17.02_688.96	2.23	0.56	1.79	7.07	8.23	0.026
	14.85_596.55	2.26	0.55	1.74	9.20	10.95	0.025
	13.81_716.64	2.22	0.78	2.46	6.97	7.66	0.028
	16.79_744.77	2.01	2.48	3.29	0.00	0.00	0.050

(*continued on next page*)

Table 1 (*continued*)

RT_m/z	VIP	NRPs		RPs		*t* test value
		Mean	SD	Mean	SD	
13.76_748.40	2.47	0.00	0.00	5.36	5.99	0.012
10.50_467.35	2.23	0.00	0.00	4.43	5.76	0.026
14.58_798.73	2.29	0.00	0.00	3.66	4.59	0.022
18.36_614.29	2.08	2.34	3.07	0.00	0.00	0.048
18.81_810.59	2.03	2.71	3.58	0.00	0.00	0.049
14.83_443.05	2.45	0.00	0.00	2.89	3.28	0.013
19.61_732.99	2.43	0.00	0.00	3.20	3.68	0.014
12.03_992.42	2.03	2.41	3.17	0.00	0.00	0.048

Bold type indicates confirmed biomarkers. Lipids are reported as a combination of RT_m/z.
NRP, not responder; PC, phosphatidylcholine; RP, responder; RT_m/z, retention time and mass/charge; SM, sphingomyelin; SD, standard deviation; PE, phatidylethanolamine; PG, phosphatidylglycerol; PS, phosphatidylserine; VIP, variable important for the projection.

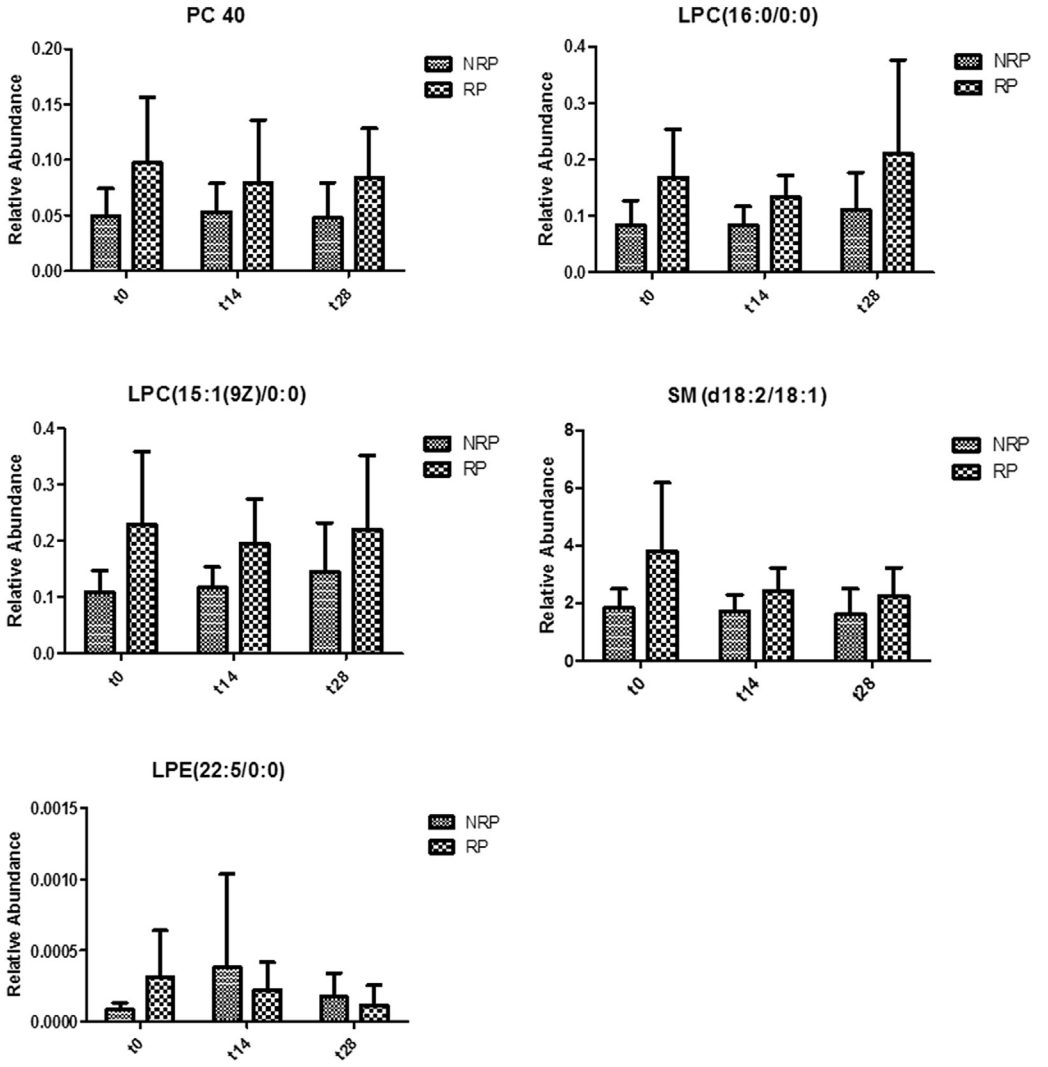

Figure 3 Histograms reporting the relative abundance of potential biomarkers in RPs and NRPs during chemoradiation therapy (CRT). Relative abundances of phosphatidylcholine (PC; 40:2), lysophosphatidylcholine (LPC;16:0/0:0), LPC (15:1 (9Z)/0:0), sphingomyelin (SM; d18:2/18:1), and lysophosphatidylethanolamine (LPE;22:5/0:0) are shown, respectively, before treatment (t0), during CRT (t14), and at the last therapy day (t28). See Fig 1 for abbreviations.

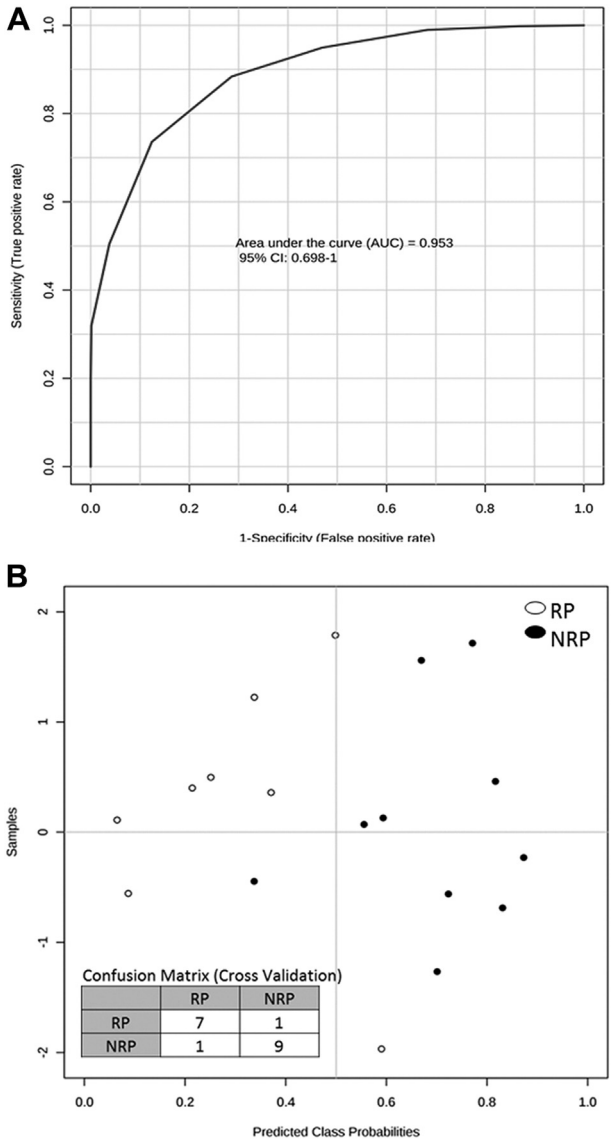

Figure 4 Predictive power of 5 validated lipids at the t0 time point. (A) Receiver operating characteristic curve generated combining the 5 validated lipids; (B) predicted class probabilities (RP or NRP) of each sample across the 100 cross-validations and the related confusion matrix generated. See Fig 1 for abbreviations.

heat map provides an overview of the different lipid signals (reported as a combination of the retention time and mass/charge [m/z]) and their relative intensity, in terms of overexpression (in red) or underexpression (in green), in RP versus NRP sera. These results help highlight the differential lipid patterns between RP and NRP sera and are summarized in Table 1.

Biomarker confirmation

To further validate the reliability of the highlighted biomarkers, an independent validation analysis was performed through targeted liquid chromatograph tandem mass spectometry. Results confirmed the lower levels in NRP of 5 differentially expressed lipids ($P < .05$) that were identified as follows: SM (d18:2/18:1) at m/z = 727.86; lysophosphatidylcholine (LPC;16:0/0:0) at m/z = 496.22; LPC (15:1(9z)/0:0) at m/z = 480.42; lysophosphatidylethanolamine (LPE;22:5/0:0) at m/z = 528.6; and PC (40:2) at m/z = 842.90. These 5 lipids were regarded as the more reliable predictive biomarkers and quantified at 14 and 28 days to evaluate their prognostic value. As shown in Fig 3, PC (40:2), the 2 LPCs, and SM confirmed their lower levels in NRP with respect to RP during the entire therapy ($P < .05$). Conversely, the levels of LPE varied during CRT. No significant difference between males and females was found in the highlighted biomarkers (data not shown).

Predictive power of lipid biomarkers

Figure 4A shows the receiver operating characteristic curve generated combining the 5 validated lipids. The area under the curve is 0.95, showing good sensitivity and specificity in discriminating between RP and NRP. The 100 cross-validations performed show the predicted class probabilities of each sample, as reported in Fig 4B, underlying the good predictivity of the proposed model ($P = .03$) in suggesting patients who may better respond to therapy.

Discussion

Predictive response biomarkers to neoadjuvant CRT in LARC could personalize treatment strategy to improve response rate and survival outcomes. In this study, we focus on serum lipids to define a discriminatory profile able to predict CRT response in LARC. Despite the small sample size analyzed, our results indicate 5 lipids that drive the separation of RP and NRP. We found that LPE (22:5/0:0), SM (d18:2/18:1), LPC (16:0/0:0), LPC (15:1(9z)/0:0), and PC (40:2) are significantly lower in NRP at t0, whereas the LPE level significantly increases in NRP during CRT. The involvement of these lipids in radioresistance may be supported by the known correlation between human phosphatidylethanolamine-binding protein 4 (hPEBP4) and inhibition of apoptosis.[14-16] Qiu et al have already demonstrated that hPEBP4 is a predictive marker of radioresistance in rectal cancer by activating Akt in a reactive oxygen species–dependent manner.[17,18]

PC (40:2) is lower in NRP compared with RP before and during treatment, probably resulting from dysregulation of choline metabolism, a known metabolic hallmark associated with oncogenesis and cancer progression.[19] Moreover, we highlighted low levels of LPCs in NRP, which is consistent with several studies that correlate higher blood LPC levels with reduced risk of cancer,[18] thus suggesting that LPCs may

represent a useful circulating biomarker for early detection of CRC.[20] The low levels of SM in NRP may be due to the high activity of SM, resulting in high levels of ceramide. Even if ceramide is involved in cell-cycle arrest, apoptosis, and senescence in CRC cells,[21,22] its degradation product, sphingosine1P, induces cell proliferation and angiogenesis and triggers cell motility.[23] Bearing in mind the limitations of this pilot study, these results provide novel insights regarding lipid metabolism in the modulation of CRT response in LARC patients. If confirmed in a more extensive clinical cohort, these biomarkers could represent a useful tool for predicting outcome as part of efforts to personalize therapy.

Supplementary data

Supplementary material for this article (http://dx.doi.org/10.1016/j.adro.2016.12.005) can be found at www.practicalradonc.org.

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