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SHORT COMMUNICATION

Is Lipidomic the Answer to the Search of a Biomarker for Organ Preservation Protocol in Head and Neck Squamous Cell Carcinoma?

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Abstract In the last decade organ preservation protocols based on chemoradiotherapy (CRT) has been showing the possibility of preserving function without jeopardizing survival for locally advanced head and neck squamous cell carcinoma (HNSCC). Still, only a percentage of the patients will benefit from this approach and, to date, no biomarkers are known to correctly predict these patients. More recently, modern mass spectrometry method has been used to determine metabolic profiles, and lipidomics, in particular, emerged as a new field of study in oncology and other diseases. This study aimed to analyze the lipid profile on saliva from patients undergoing to a prospective, single center, open-label, non-randomized phase II trial for organ preservation on HNSCC. The lipid analysis was performed using matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF-MS). Multivariate statistical analyses based on principal component analysis and orthogonal partial least square-discriminant analysis were applied to MALDI-TOF-MS data to visualize differences between the lipid profiles and identify potential biomarkers. The results assisted on distinguishing complete responders from non-responders to the treatment protocol. In conclusion, we demonstrated that a group of lipids is differentially abundant in saliva from HNSCC patients submitted to an organ preservation protocol, being able to differentiate responders from non-responders. These results suggest the potential use of lipid biomarkers to identify patients who may benefit from this treatment. Also, we showed that saliva testing might be routinely used in clinical practice, for being a non-invasive alternative to blood testing, besides inexpensive and easy to obtain.

Keywords Head and neck cancer · Induction chemotherapy · Lipidomics · Mass spectrometry · Biomarker · Response to treatment

Introduction

Head and neck squamous cell carcinoma (HNSCC) is an epithelial malignancy that arises in the upper aero digestive tract. HNSCC is the fifth most common type of cancer affecting approximately 600.000 patients per year, worldwide [16]. The most important risk factors are tobacco use, alcohol consumption and the human papillomavirus (HPV) infection [11]. The management for HNSCC is often complicated, involving different possibilities combining surgery, radiotherapy and chemotherapy [3]. Moreover, disease prognosis significantly depends on the site and stage of the primary tumor, and survival rate remains poor for patients with locally advanced tumors (stage III and IV) [12]. In the last decade organ preservation protocols based on chemoradiotherapy (CRT) has been showing the possibility of preserving function without jeopardizing survival for locally advanced HNSCC patients showed that only 30-50% of them survives more than 3 years after diagnosis [1, 4]. Latter, the introduction of induction

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chemotherapy (IC) followed by CRT arose as an approach to HNSCC and some studies showed higher rates of complete response, less distant recurrence and higher larynx preservation rate in patients undergoing IC [7, 8]. However, not all patients benefit from the organ preservation protocols. Once it is an aggressive treatment, associated with many comorbid conditions, it would be of paramount important to identify potential molecular biomarkers that can predict response to treatment, as well prognosis, and could be evaluated before treatment starts, allowing for a personalized treatment.

In the last years, the "omics" technologies allowed detailed genetic, epigenetic and protein profile characterization of different human diseases. More recently, modern mass spectrometry approaches have been used to determine metabolic profiles, and lipidomics, in particular, emerged as a new field of study in oncology and other diseases. Lipids can differ between cell and tissue types, as well in response to physiological changes. They are essential cellular components, have many functions and regulate several biological processes by acting as signaling molecules and precursors for second messengers. So, lipids can regulate signal transduction pathways, cell proliferations and apoptosis, and others cell functions [5]. Different groups demonstrated that normal tissues have a different lipid composition from tissues affected by some diseases [15, 18, 19], and it indicates that changes in abundances of particular lipids are a consequence of changes in genetic status, epigenetic regulation, protein expression and post-translational modifications. In fact, malignant cells may differ from healthy ones by their lipid metabolism; increased lipogenesis is a common feature of most solid tumors and may also have an effect to the chemosensitivity of the cells [6]. Thus, it has been suggested that lipidomics can work as a tool for biomarker discovery for disease diagnosis and response to treatment.

Methods

A prospective, single center, open-label, non-randomized phase II trial was conducted by Head and Neck, Clinical Oncology and Pathology Departments of Barretos Cancer Hospital to evaluate the efficacy, safety and tolerance of IC with cisplatin and paclitaxel followed by concurrent CRT with cisplatin in patients with locally advanced HNSCC (stage III and IV) and showed that this regimen is safe, well-tolerated, and has a high overall response rate [14]. Sixty patients were included in this clinical trial and saliva from 55 of them was collected in the moment of diagnosis (before the patients have been submitted to any treatment with chemo- or radiotherapy). After informed consent was obtained from all participants, these samples were used in the present study to determine the lipidomic profiles and evaluate if patients with complete response vs. non-responders after the organ preservation protocol have specific lipid signatures that could predict response to treatment. The lipid extraction was performed as described by Bligh and Dyer [2], followed by lipidomic profiling analysis by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS). Mass spectra were acquired in the positive ion mode using a quadrupole-TOF (Q-ToF) Premier (Synapt HDMS) mass spectrometer (Waters, Manchester, UK) equipped with a 200 Hz solid-state laser in the *m/z* 700-1200 range, in the reflectron mode. Typical operating conditions were: laser energy 250 a.u., sample plate 20 V and the *Trap* and *Transfer* collision energies were 6 and 4 V, respectively (QTOF-MS mode). The mass spectra processing of each sample was performed by MarkerLynx 4.1 software (Waters, Manchester, UK) and exported to Microsoft® Excel® for further principal component analysis (PCA) and orthogonal partial least square discriminant analysis (OPLS-DA) by MarkerLynx™ XS

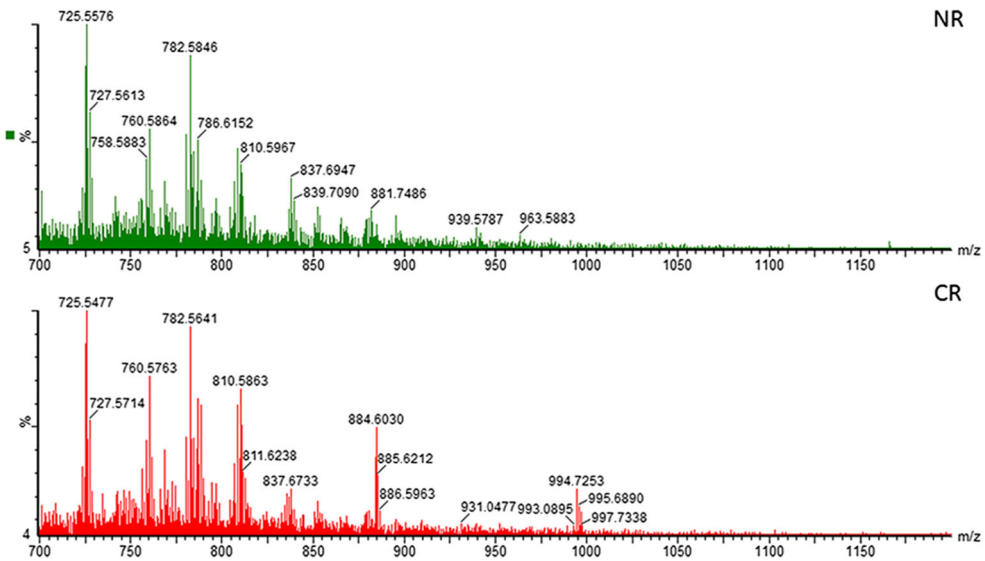

Fig. 1 MALDI-MS representative spectrum for each group of patients with complete response to treatment (CR) and the group of non-responders patients (NR). The y-axis shows relative abundances whereas the x-axis shows m/z values

(Waters, Manchester, UK). The method parameters were as follows: mass tolerance = 0,5 Da, baseline noise = 50 and intensity threshold (count) = 1000 with deisotope data. The S-plot of the OPLS-DA analysis provided a list of ions responsible for the differences between the groups, and the lipid subclasses of these ions were searched in the Human Molecular Database (HMDB) (http://www.hmdb.ca). A mass tolerance of 0.1 Da was adopted and the maximum mass error considered was ≤50 ppm for all the identified ion masses.

Based on prospective evaluation during the clinical trial, treatment response was accessed by RECIST 1.1 criteria. Then, they were divided into two groups, according to their response after CRT: 34 patients presented complete response (CR) and 23 had a partial response, stable or disease progression and were considered non-responder (NR); 3 patients died

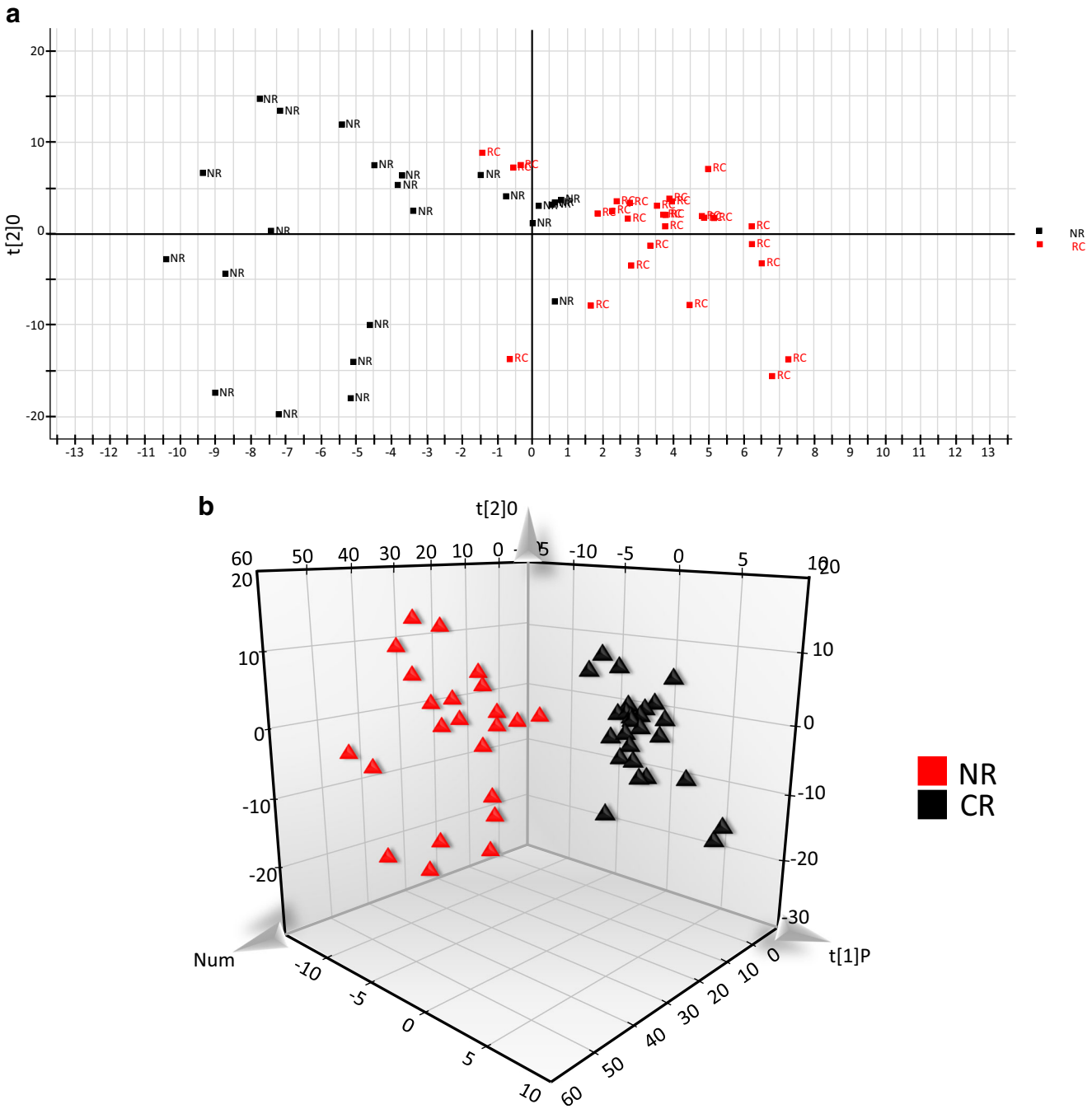

Fig. 2 **a** PCA 2D shows groups separation considering the scores of PC1 *vs*. PC2. The black points show the non-responders group by individual PCs; the red points show the responders patients. **b** OPLS-DA 3D Plot shows complete groups separation based on the lipidomics analysis of responders (black triangles) *vs*. non-responders (red triangles)

before the end of CRT treatment or abandoned the treatment [14].

Results

On the first lipidomic analysis, it was possible to determine the representative spectra of lipids presented exclusively in each group of patients (CR *vs*. NR) (Fig. 1). Multivariate statistical analyses, such as principal component analysis (PCA) and orthogonal partial least square-discriminant analysis (OPLS-DA), were carried out on preprocessed MALDI-TOF-MS dataset, including considering the *m/z* values, to observe the difference between the groups lipid profiles and identify potential lipid biomarkers. The 2D PCA plot of samples from patients with complete response and non-response showed that most part of the samples from the same group are together in the graph, suggesting that lipid profile can separate these two group of patients (Fig. 2a). Next, the OPLS-DA showed a clear separation between responders and non-responders patients (Fig. 2b). The analysis of which lipids were differentially abundant for the responder and non-responder groups identified 50 molecules. Of them, 25 phosphatidylethanolamines (PE), 22 phosphatidylcholines (PC) and 1 sphingomyelin (SM). Also, 1 intermediate in cholesterol biosynthesis (4a-Carboxy-4b-methyl-5a-cholesta-8,24-dien-3b-ol) and 1 steroid derivative involved in steroid biosynthesis (4a-Methylzymosterol-4-carboxylic acid).

Discussion

These results showed that salivary lipid profile of patients with HNSCC could predict the response to the organ preservation protocol. Some studies previously described lipid profiling alterations for various human diseases [15, 18, 19], suggesting that key lipids could indicate a disease state, as well diagnosis and prognosis. A few numbers of reports analyzed lipid profile in human cancer. Hilvo et al. [6] showed that increased oleic acid content in the serum triacylglycerols is associated with poorer response to neoadjuvant chemotherapy (with epirubicin, cyclophosphamide → docetaxel *vs.* epirubicin, cyclophosphamide → docetaxel/capecitabine *vs.* epirubicin, cyclophosphamide → docetaxel → capecitabine) in breast cancer patients. Using Hela Cells, Li and Yuan [9] described discriminatory phospholipid biomarkers between the control and paclitaxel-treated cells. To HNSCC, Wang et al. [17] performed metabolomics analysis in saliva and identified potential biomarkers to early diagnosis in oral squamous cell carcinoma, whereas Saddoughi et al. [13] showed that serum C_{18}-ceramide elevation might be a novel biomarker for chemotherapy response with gemcitabine/doxorubicin to recurrent or metastatic HNSCC patients. Recently, a report found that levels of cholesterol, low-density lipoprotein (LDL), apolipoprotein A, and apolipoprotein B, were significantly lower in patients with HNSCC than in control subjects and higher lipoprotein (a) was associated with poorer prognosis for these patients [10].

Conclusion

In conclusion, we demonstrated that a group of lipids are differentially abundant in saliva from HNSCC patients submitted to an organ preservation protocol, being able to discriminate responders from non-responders after treatment. These results suggest the potential use of lipidomics as a tool for biomarkers identification to discriminate patients who may benefit from this treatment. Also, we showed that saliva testing could be routinely used in clinical practice, as a non-invasive alternative to blood testing and due its easy collection properties and low costs for the hospitals and patients.

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Compliance with Ethical Standards

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

Conflicts of Interest Statement The authors declare that they have no conflict of interest.

References

1. Argiris A, Karamouzis MV, Raben D, Ferris RL (2008) Head and neck cancer. Lancet 371(9625):1695–1709
2. Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37(8):911–917
3. Denaro N, Russi EG, Adamo V, Merlano MC (2014) State-of-the-art and emerging treatment options in the management of head and neck cancer: news from 2013. Oncology 86(4):212–229
4. Haddad RI, Shin DM (2008) Recent advances in head and neck cancer. N Engl J Med 359(11):1143–1154
5. Hannun YA, Obeid LM (2008) Principles of bioactive lipid signalling: lessons from sphingolipids. Nat Rev Mol Cell Biol 9(2):139–150
6. Hilvo M, Gade S, Hyotylainen T, Nekljudova V, Seppanen-Laakso T, Sysi-Aho M et al (2014) Monounsaturated fatty acids in serum triacylglycerols are associated with response to neoadjuvant chemotherapy in breast cancer patients. Int J Cancer 134(7):1725–1733
7. Holsinger FC, Lin HY, Bassot V, Laccourreye O (2009) Platin-based exclusive chemotherapy for selected patients with squamous cell carcinoma of the larynx and pharynx. Cancer 115(17):3909–3918
8. Lefebvre JL, Chevalier D, Luboinski B, Kirkpatrick A, Collette L, Sahmoud T (1996) Larynx preservation in pyriform sinus cancer: preliminary results of a European Organization for Research and

Treatment of Cancer phase III trial. EORTC Head and Neck Cancer Cooperative Group. J Natl Cancer Inst 88(13):890–899
9. Li X, Yuan YJ (2011) Lipidomic analysis of apoptotic hela cells induced by Paclitaxel. Omics: J Integr Biol 15(10):655–664
10. Li G, Da M, Zhang W, Wu H, Ye J, Chen J et al (2016) Alteration of serum lipid profile and its prognostic value in head and neck squamous cell carcinoma. Journal of oral pathology & medicine: official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology 45(3):167–172
11. Marur S, D'Souza G, Westra WH, Forastiere AA (2010) HPV-associated head and neck cancer: a virus-related cancer epidemic. Lancet Oncol 11(8):781–789
12. Pignon JP, le Maitre A, Maillard E, Bourhis J, Group M-NC (2009) Meta-analysis of chemotherapy in head and neck cancer (MACH-NC): an update on 93 randomised trials and 17,346 patients. Radiotherapy and oncology: journal of the European Society for Therapeutic Radiology and Oncology 92(1):4–14
13. Saddoughi SA, Garrett-Mayer E, Chaudhary U, O'Brien PE, Afrin LB, Day TA et al (2011) Results of a phase II trial of gemcitabine plus doxorubicin in patients with recurrent head and neck cancers: serum C(1)(8)-ceramide as a novel biomarker for monitoring response. Clinical cancer research: an official journal of the American Association for Cancer Research 17(18):6097–6105
14. de Souza Viana L, de Aguiar Silva FC, Andrade Dos Anjos Jacome A, Calheiros Campelo Maia D, Duarte de Mattos M, Arthur Jacinto A et al (2016) Efficacy and safety of a cisplatin and paclitaxel induction regimen followed by chemoradiotherapy for patients with locally advanced head and neck squamous cell carcinoma. Head Neck 38(1):E970–E980
15. Stegemann C, Drozdov I, Shalhoub J, Humphries J, Ladroue C, Didangelos A et al (2011) Comparative lipidomics profiling of human atherosclerotic plaques. Circ Cardiovasc Genet 4(3):232–242
16. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A (2015) Global cancer statistics, 2012. CA Cancer J Clin 65(2):87–108
17. Wang Q, Gao P, Wang X, Duan Y (2014) The early diagnosis and monitoring of squamous cell carcinoma via saliva metabolomics. Sci Rep 4:6802
18. Wood PL (2012) Lipidomics of Alzheimer's disease: current status. Alzheimers Res Ther 4(1):5
19. Zhao C, Mao J, Ai J, Shenwu M, Shi T, Zhang D et al (2013) Integrated lipidomics and transcriptomic analysis of peripheral blood reveals significantly enriched pathways in type 2 diabetes mellitus. BMC Med Genet 6(1):S12