WP 399

Oncofinder: a novel platform for screening benignant nevi from melanomas based on lipid phenotype using mass spectrometry and machine learning

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1. Overview

- We demonstrate the initial evaluation of a prototype dual polarity benchtop MALDI-TOF mass spectrometer for screening melanomas from benignant nevi based on the detected lipid profiles. Melanoma is the most serious form of skin cancer due to early metastasis leading to death.
- In the limited number of samples tested, the workflow demonstrated 100% sensitivity for melanoma classification.
- The use of a simple MALDI-TOF MS offers several advantages compared with other MS techniques (e.g. robustness, ease of use, throughput, low cost) for this type of routine application.

2. Introduction

Oncofinder is a Research-Use Only (RUO) platform that is being developed for the diagnosis, staging and prognosis of melanoma using the lipid fingerprints acquired from tissue or liquid biopsies. A panel of lipid biomarkers allows the rapid and accurate screening of benignant nevi from melanomas by studying the lipid fingerprint using mass spectrometry and artificial intelligence (see figure 1).

This technology circumvents the limitations inherent to current methodologies, based on dermoscopic, histopathological and surrogate biomarkers, reducing the laboratory work and providing a faster and easier diagnosis. Moreover, the specificity of the lipid biomarkers together with the power of mass spectrometry and machine learning confer to the Oncofinder platform strong prognostic capabilities not only to detect melanoma but also to determinate their malignant potential. However, the high complexity, size and cost of the mass spectrometers limits its implementation in anatomic pathology analytical services.

In this work, we evaluate a prototype dual polarity linear benchtop MALDI-TOF mass spectrometer (Shimadzu) for the generation of the lipid profiles. A preliminary screening of a collection of human biopsies of nevi and melanomas demonstrated a high potential for screening melanoma from benignant biopsies with a sensitivity near 100% with a representative collection of samples.

MS+	Reference test		
	Melanoma	Nevi	**Total**
Oncofinder Melanoma	51	3	54
Nevi	0	21	21
Total	51	24	**75**

Sensitivity: 100.0 % Specificity: 87.5 % Accuracy: 96.0%

MS-	Reference test		
	Melanoma	Nevi	**Total**
Oncofinder Melanoma	78	24	102
Nevi	0	36	36
Total	78	60	**138**

Sensitivity: 100.0 % Specificity: 60.0 % Accuracy: 82.6%

Figure 1: Previous classification results using the Oncofinder platform (MALDI-Orbitrap data)

3. Methods

Skin biopsies from nevi and melanoma were homogenized in a reaction medium, mixed with MALDI matrices in a suitable sample/matrix ratio and deposited on a stainless steel MALDI target. 2-mercaptobenzothiazole (MBT) was used for positive-ion mode, 1,5-diaminonaphthalene (DAN) and 9-aminoacridine (9-AA) were used for negative-ion mode. Spectra were acquired and calibrated both in positive (480-1000 Da) and negative (550-1000 Da) ion modes on a prototype dual polarity linear benchtop MALDI-TOF (Shimadzu). The exported data were analyzed and classified into 2 classes (nevus – melanoma) by the Oncofinder software which issues an automated diagnostic based on machine learning algorithms using a specific lipid biomarker database.

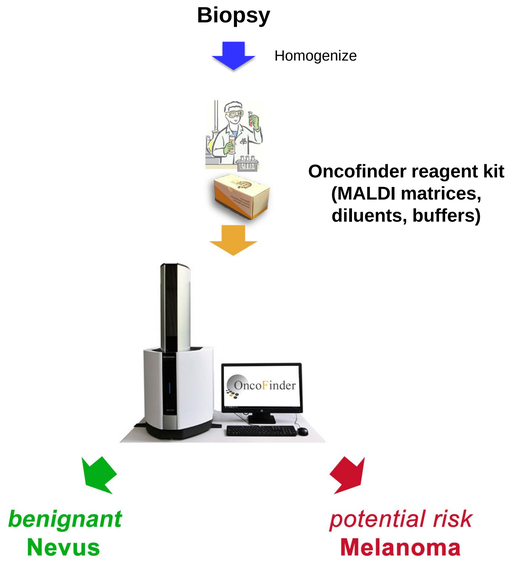

Figure 2: Oncofinder workflow: Skin biopsies are homogenized and the extracted lipids are prepared for MALDI-MS analysis using 2-mercaptobenzothiazole (MBT) for positive-ion mode and 1,5-diaminonaphthalene (DAN) or 9-aminoacridine (9-AA) for negative mode analysis. Following MALDI-MS analysis, the data are exported and unsupervised classification is performed using the Oncofinder software. Examples of the results are shown in Figures 4 and 5.

4. Results

Example positive and negative mode MALDI-MS spectra are shown in Figure 3.

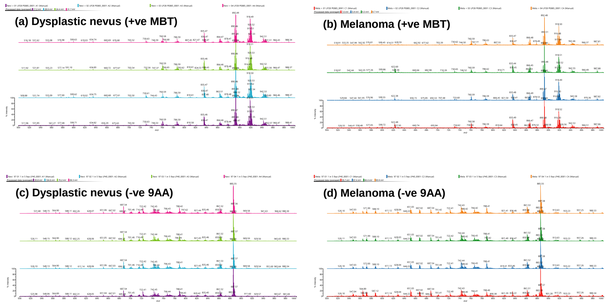

Figure 3: Example MALDI-MS spectra for nevus and melanoma samples. (a) and (b) were acquired in positive ion mode using MBT matrix, (c) and (d) were acquired in negative ion mode using 9-AA matrix.

The lipid fingerprint, comprising the simultaneous detection of >50 lipids, provides great sensitivity and specificity to the test, allowing an accurate classification of malignant tumors. Together with the Oncofinder software, this rapid test performs a preliminary screening of biopsies, providing a selection of samples that require further anatomopathological confirmation. Example results are shown in figures 4 and 5.

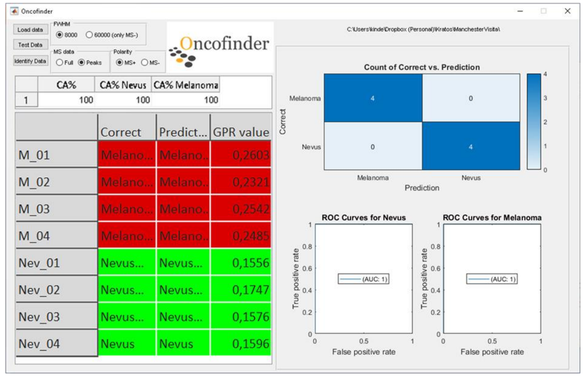

Figure 4: Reported results following data analysis using the Oncofinder software. The above example shows identifications obtained using the positive ion mode spectra acquired with the benchtop linear MALDI-TOF mass spectrometer. Red = melanomas correctly identified, green = nevus correctly identified.

In negative ion mode, the prediction achieved a 100% of sensitivity for detecting melanoma with an accuracy of 62.5% due to included nevus (see figure 5). Moreover, the GPR value obtained was close to the threshold for two of the nevi classified as melanoma, suggesting that the accuracy could be substantially increased with further optimization.

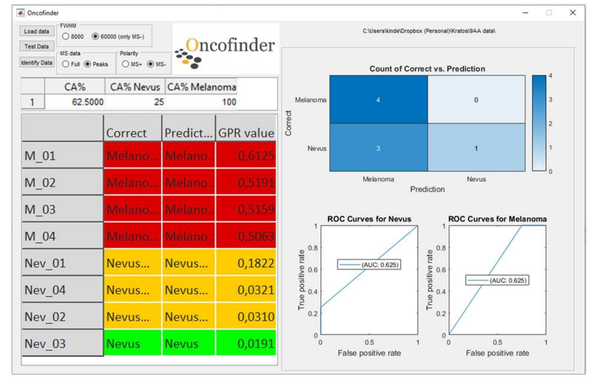

Figure 5: Reported results following data analysis using the Oncofinder software. The above example shows identifications obtained using the negative ion mode spectra acquired with the benchtop linear MALDI-TOF mass spectrometer. Red = melanomas correctly identified, green = nevus correctly identified, orange = ambiguous identification.

For this type of application, the sensitivity of the test for detecting melanomas is the most important factor i.e. it is vital that all cases of melanoma are correctly identified. Nevi samples which are *incorrectly* assigned as melanomas (i.e. false positives) result in additional cases which would require further confirmation by a specialist. The most important aspect as a screening test is that all melanomas are correctly identified, even if this includes some misidentified nevi, thereby reducing the number of samples to be analyzed using histopathological techniques.

5. Conclusions

- Here we present the first application of a benchtop linear MALDI-TOF mass spectrometer for screening melanoma, which uses a collection of lipid biomarkers and accurate AI algorithms for identification.
- The Oncofinder platform provides a diagnosis of the benignity of skin samples and the potential risk associated, with nearly 100% sensitivity for melanoma. It is hoped that the lower specificity of the test using negative ion mode data can be improved with further optimization of the algorithm parameters.
- Results suggest that this technology could provide a faster, cost effective and easier diagnosis to anatomic pathology analytical services, although a further study with a larger collection of samples must be required