The Association for Mass Spectrometry: Applications to the Clinical Lab

Mass spectrometric imaging of cysteine rich proteins in human skin

Tomas Do[1], Lucie Vanickova[1,2], Roman Guran[1,2], Sandor Kollar[3], Gabriella Emri[4], Sona Krizkova[1,2], Zbynek Heger[1,2], Ondrej Zitka[1,2,5], Vojtech Adam[1,2,5]

[1]Mendel University, Brno, Czech Republic

[2]Central European Institute of Technology, Brno University of Technology, Brno, Czech Republic

[3]Kenézy Gyula County Hospital, Debrecen, Hungary

[4]University of Debrecen, Debrecen, Hungary

[5]Central European Institute of Technology, Mendel University, Brno, Czech Republic

Introduction

Looking insight pathological processes, metallothioneins (MTs) are considered to be potential biomarkers for monitoring of a development of various types of diseases, such as cancer. The early identification of the MTs in biological tissues could be important tool for the estimation of appropriate clinical therapy.

Objectives

The aim of this study was to investigate the application of matrix assisted laser desorption/ionization mass spectrometry imaging (MALDI MSI) together with immunohistochemical analyses (IHC) using MT-1/2 antibody for MT detection in biopsy specimens of human skin.

Methodology

Formalin-fixed paraffin-embedded (FFPE) tissue blocks of various types of skin cancers were selected for analysis. Normal skin was derived from an 83-year-old female patient, who had a keratoacanthoma on her forearm. The resection margin free of any tumour cells was used for the study. Basal cell cancer (basalioma solidum et cysticum) was localized on the shoulder of a 61-year-old male patient. Superficially spreading malignant melanoma with Breslow tumour thickness 2.76 mm, Clark level III, 0–2/hpf mitotic activity (pT3a) was removed from the thigh of a 47-year-old male patient. Grade 2 cutaneous squamous cell cancer of an 86-year-old female patient was localized on her nose. Melanocytic nevus was removed from the chest of a 45-year-old female patient. FFPE tissues were histologically characterized, analyzed by MALDI MSI, and immunohistologically stained.

Results

Principal component analyses of MSI data revealed differences in the peptide/protein profiles separating healthy skin from the carcinoma specimens. Statistically significant ion peaks at *m/z* 6038, 6300, 6676, and 7026 were more frequently detected in squamous cell carcinoma (SCC), basal cell carcinoma (BCC) and melanoma. Using IHC, we found that MT-1/2 was significantly higher in SCC and melanoma compared to healthy skin. Surprisingly, significantly low levels of MT-1/2 were found in BCC.

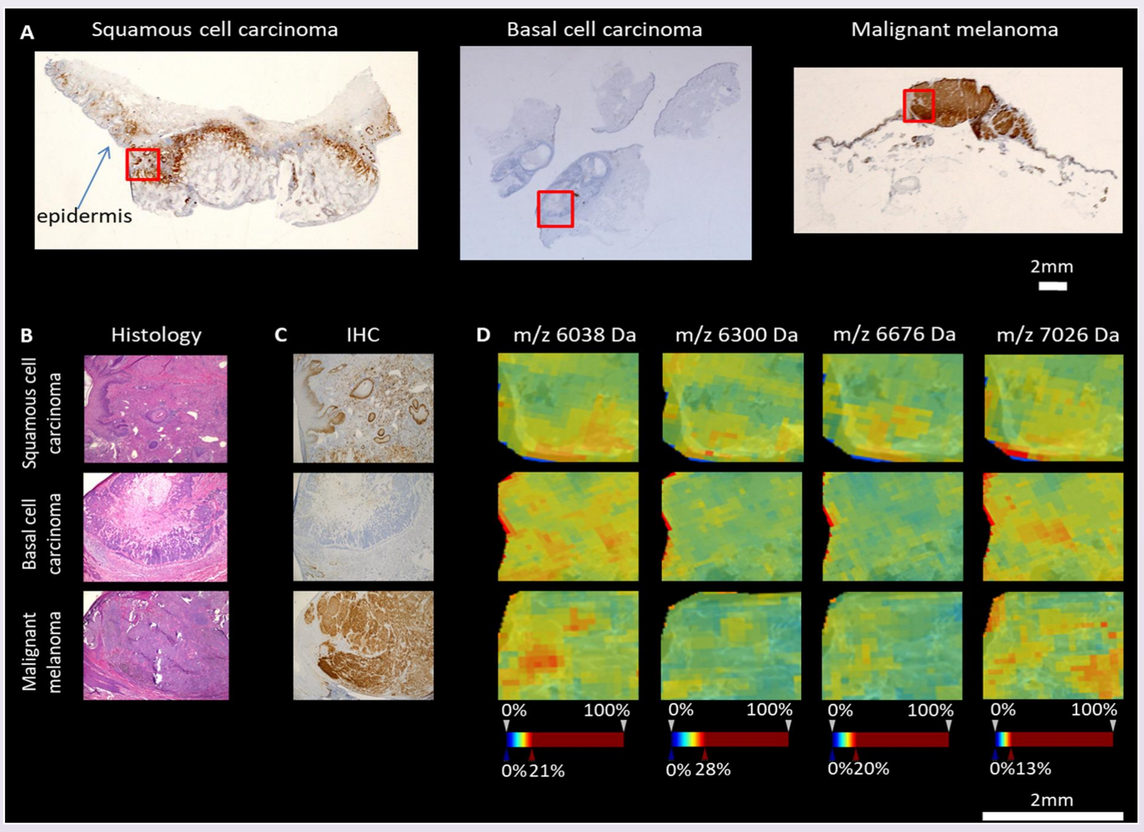

Figure 1: Immunohistochemical (IHC), histological and MSI analysis of squamous cell carcinoma (SCC), basal cell carcinoma (BCC) and malignant melanoma (MM). (A) Representative photomicrograph showing increased (brown colour) or decreased MT-1/2 immunoreactivity in specific skin tissues. (B) Magnified histological photomicrograph showing the selected area (red rectangle) of the particular tissue type. (C) Magnified photomicrograph showing increased (brown colour) or decreased MT-1/2 immunoreactivity in specific skin tissues in the selected area (red rectangle). (D) Protein maps for ion peaks at *m/z* 6038, 6300, 6676 and 7026 overexpressed in SSC, BCC and MM in the selected area (red rectangle), generated as "[*m/z*] ± 10 Da". Scale bar = 2 mm (Vanickova et al. 2019).

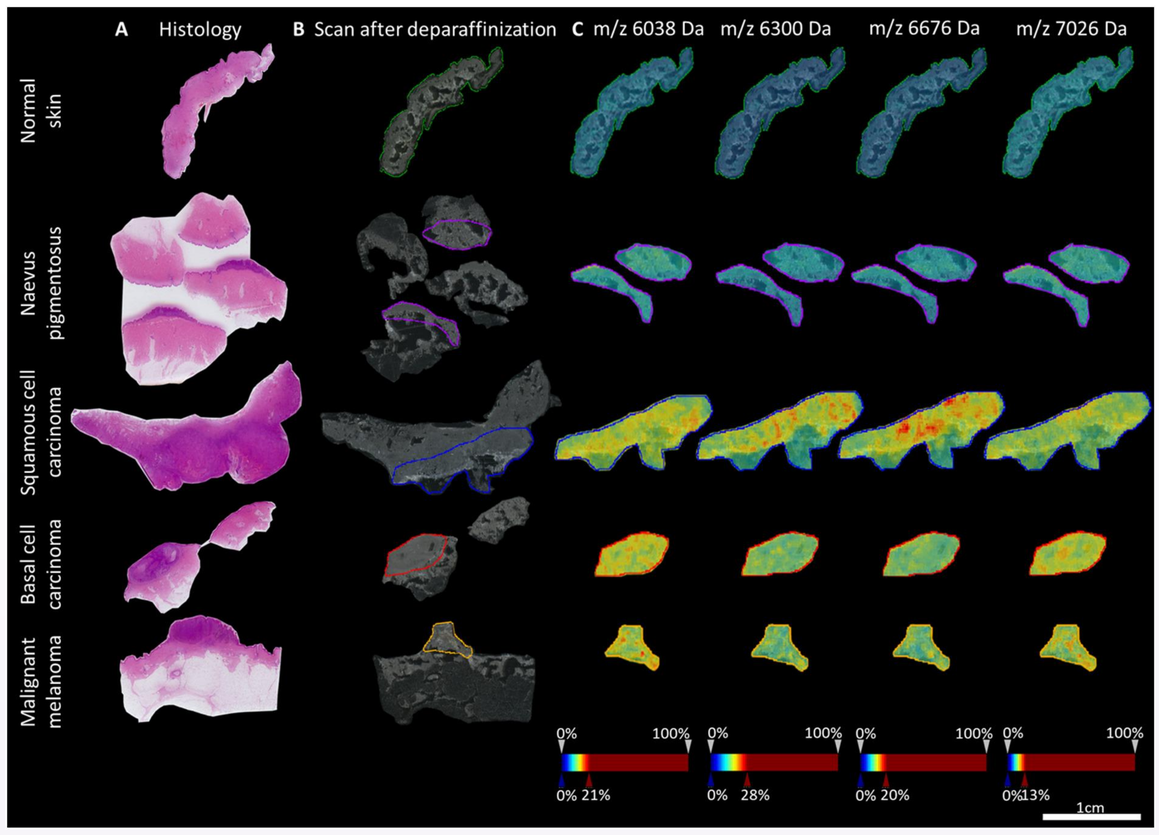

Figure 2: Histological and MSI analysis of five different skin tissues, namely normal skin, naevus pigmentosus, squamous cell carcinoma (SCC), basal cell carcinoma (BCC), and malignant melanoma (MM). (A) Representative histological photomicrograph showing the particular tissue type. (B) Scanned pictures of the particular tissue type after the deparaffinization together with selected regions of interest (ROIs) used for MSI analysis. (C) Protein maps for ion peaks at *m/z* 6038, 6300, 6676 and 7026 generated as "[*m/z*] ± 10 Da", scale bar = 1 cm (Vanickova et al. 2019).

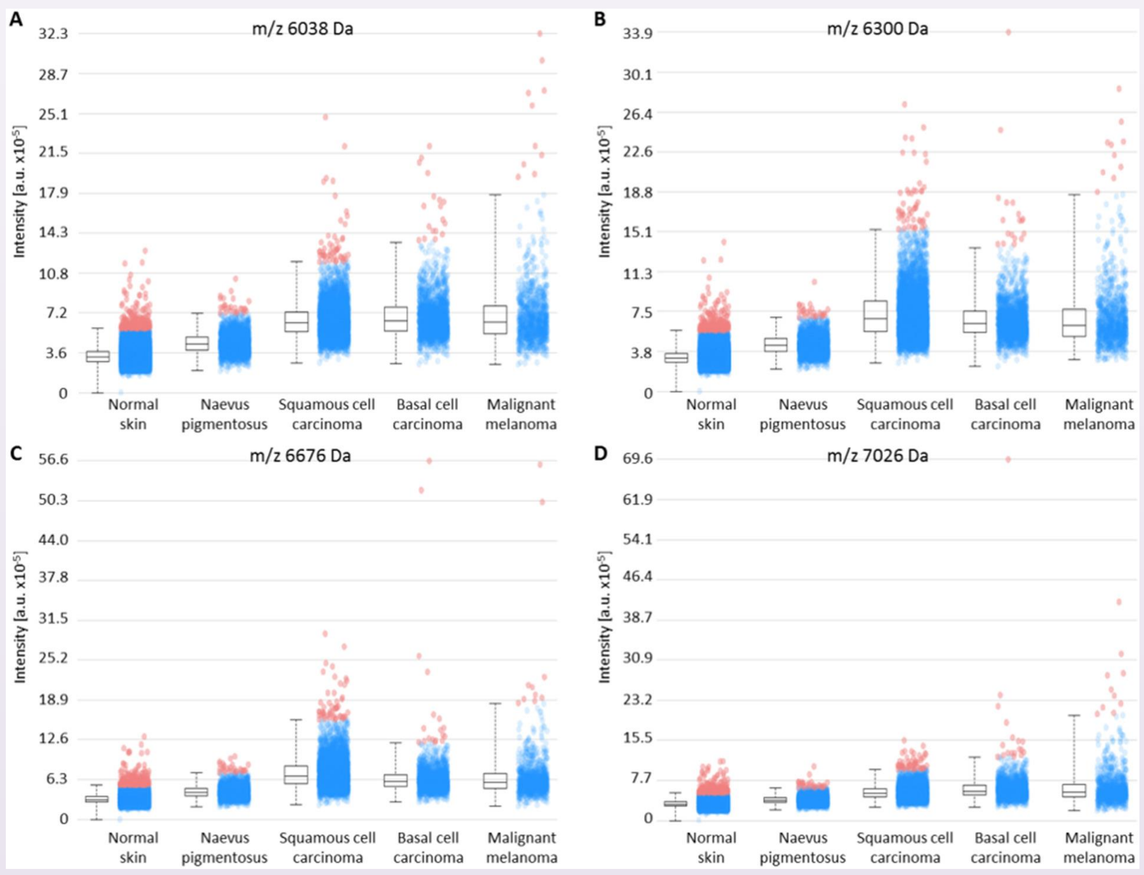

Figure 3: Intensity box plots of selected protein *m/z* values (A) 6038, (B) 6300, (C) 6676 and (D) 7026 in five different skin tissues (Vanickova et al. 2019).

Acknowledgements

This research has been financially supported by the Internal Grant Agency of Mendel University in Brno (project No. AF-IGA-2018-tym005), by the Ministry of Education, Youth and Sports of the Czech Republic under the project CEITEC 2020 (LQ1601). VA wishes to express his thanks to the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 759585). LV was supported by project 6SA17676 that received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie and which is co-financed by the South Moravian Region under grant agreement No. 665860

Conclusion

The results indicate important role of MTs in melanoma occurrence and progression. There are hidden processes associated with MTs based on differences of the occurrence of the MS peaks, which could be associated with cycling of MTs isoforms. In conclusion, our results contributed to the basic knowledge on protein distribution in histologically specified regions in skin cancer tissues.