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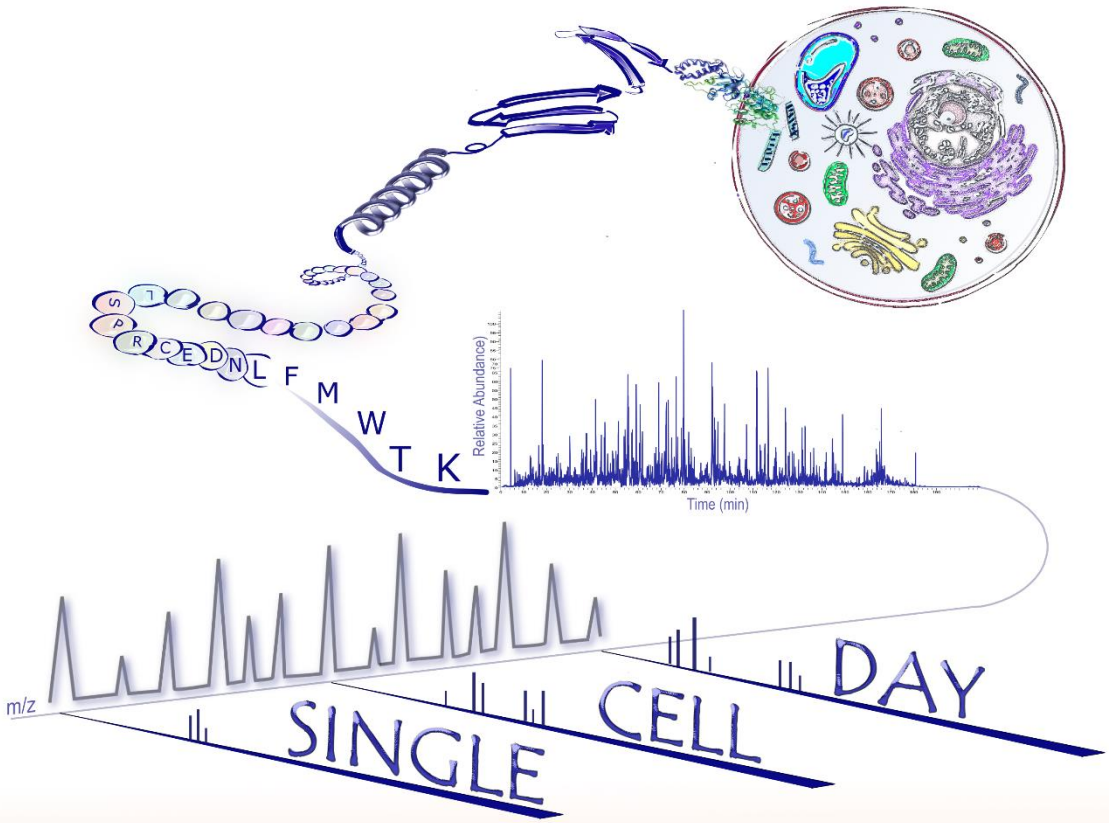

Parma, 12 October 2022

Hybrid event: Zoom platform & Centro Santa Elisabetta, Parco Area Delle Scienze University of Parma

Organizing Commettee

Barbara Pioselli - *Chiesi Farmaceutici*
Barbara Montanini - *Università di Parma*
Silvia Catinella - *Chiesi Farmaceutici*
Andrea Armirotti - *IIT Genova*
Claudio Iacobucci - *Università dell'Aquila*
Lolita Piersimoni - *Chiesi Farmaceutici*
Simone Sidoli - *Albert Einstein College of Medicine*

Invited speakers

Claudia Ctortecka - *Broad Institute of MIT and Harvard*
Ronald Cutler - *Albert Einstein College of Medicine*
Karl Mechtler - *Research Institute of Molecular Pathology*
Francesca Ruscitti - *Chiesi Farmaceutici*
Erwin Schoff - *Technical University of Denmark*
Nikolai Slavov - *Northeastern University*

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THE INFANCY OF SINGLE CELL ANALYSIS: PROTEOMIC PROFILE OF DIFFERENT PHENOTYPES OF HUMAN MONOCYTE-DERIVED MACROPHAGES

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Abstract

Tissue macrophages play a key role in many aspects of human physiology and pathology. These cells are heterogeneous both in term of morphology and function. As an example, heterogeneity has been reported within the atherosclerotic lesions where distinct populations exert opposite functions driving plaque progression or stability. Tissue macrophages are not easily obtained, and blood-derived monocytes are largely used as surrogate model. We previously reported that human macrophages spontaneously differentiated from adherent monocytes show two dominant subsets, distinct for morphology (spindle and round) and functions.

The aim of this study was to evaluate the intracellular proteome of these two macrophage subsets by means of a microproteomic workflow properly set up to quantify proteins from a minimal number of morphotypically heterogeneous cells in culture isolated by laser capture microdissection.

We report two distinct proteomic profiles that distinguish round from spindle macrophages. In particular, differential abundances were observed for proteins involved in membrane traffic regulation, lipid handling, efferocytosis, and protection against stress conditions.

Results from this study reinforce and extend previous data on the functional and antigenic profile of these macrophage phenotypes strengthening the suitability of our model to focus on macrophage heterogeneity and the benefits of single cell analysis.

Keywords: Macrophage heterogeneity; Laser capture microdissection; Label-free quantitative mass spectrometry

METABOLITE PROFILING AND BIOACTIVITIES OF LEAVES, STEMS AND FLOWERS OF RUMEX USAMBARENSIS (DAMMER) DAMMER, A TRADITIONAL AFRICAN MEDICINAL PLANT.

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Abstract

The comprehensive identification of secondary metabolites represents a fundamental step for the assessment of bioactivities and pharmacological properties of traditional herbal drugs. *Rumex usambarensis* (Dammer) Dammer has been described as a multipurpose remedy in different African traditional pharmacopoeias, but its phytochemical profile has not been properly investigated. Herein we report a high throughput metabolomic screening, based on ultra-high performance liquid chromatography-travelling wave ion mobility spectrometry quadrupole time-of-flight (UHPLCTWINS-QTOF), which was performed for the first time on different *R. usambarensis* plant parts. By applying high-resolution mass spectrometry-based metabolomics and chemometric analysis, a complete discrimination of different aerial parts was obtained, with the annotation of 153 significant metabolites in leaves, stems, and flowers, suggesting an easy authentication and discrimination route. Phytochemical data were correlated to antimicrobial and antioxidant properties. Flavonoids, benzopyranes, chromones and xanthones derivatives, along with a richer phytocomplex, might be responsible for the stronger bioactivities obtained from flowers.

Keywords: Traditional Healers Knowledge, Medicinal Plants, Untargeted Metabolomics, UHPLCTWINS-QTOF

MASS SPECTROMETRY-BASED PROTEOMIC PROFILING OF SKIN ECCHYMOSIS AS INSIGHT TOOL FOR FORENSIC INVESTIGATION

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Abstract

In forensic medicine, assessment of the lapse time between injuries and death is a well-known issue to be addressed. In this context, mass spectrometry (MS)-based proteomics recently drew attention of pathologists and forensic scientists due to its potential to strengthen scientific evidence in legal cases; moreover, differential proteomics of biological samples under diverse physiological states could lead to the identification of new biomarkers [1-3].

In this work, a high-throughput bottom-up proteomic approach based on nano-liquid chromatography/high-resolution mass spectrometry (nanoLC-HRMS) has been applied to fullthickness human skin for the differential analysis of normal and ecchymotic tissue, with the aim of identifying new biomarkers for bruise characterization and dating.

First, proteomic profiling of full-thickness skin tissues was acquired through an efficient protein extraction strategy under denaturing conditions, tryptic digestion, nanoLC-HRMS analysis, and data processing. About 2000 proteins deriving from 18 individuals pooled in 5 samples were identified from ecchymotic\non-ecchymotic skin pairs. The developed method showed excellent reproducibility in terms of the LFQ (label-free quantitation) intensity of the signals in independent replicates performed on the same pooled samples; in addition, the number of identified proteins on replicates of the same samples never deviate more than 2.5% and 6%, respectively, and the

Pearson correlation coefficients was always higher than 95%. Analyses allowed to identify six proteins which are present only in the ecchymotic tissue: Glycophorin-A, c-Myc-binding protein, protein unc-13-homolog C, beta-adducin, hemoglobin subunit gamma-2, and proteasomal ubiquitin receptor ADRM1. A particular focus was given to seven cases with known age of ecchymosis, where Glycophorin-A was exclusively found in ecchymotic tissues; this result was confirmed by Western blotting analysis on pooled samples.

In conclusion, for the first time, a mass spectrometry-based approach was developed for the proteomic profiling of skin ecchymosis, allowing not only to confirm the presence of proteins previously associated with wound healing, but also to identify proteins, in particular Glycophorin-A, exclusively present in vital ecchymotic skin lesions. The present work also showed the potentialities of MS proteomics for wound dating purposes, permitting to draw up a shortlist of proteins possibly related to wound age; however, the assessment of these proteins as marker for wound dating will require further studies.

[1] V.-A. Duong, J.-M. Park, H.-J. Lim, H. Lee, *Appl. Sci.* 2021, *11*, 3393

[2] G. J. Parker, H. E. McKiernan, K. M. Legg, Z. C. Goecker, *Forensic Sci. Int. Genet.*, 2021, *54*, 102529 [3] N. Li, Q. Du, R. Bai, J. Sun, *Forensic Sci. Res*. 2020, *5*, 15-24

IMaSS Single Cell Day 2022 -Hybrid event

Parma, 12-10-2022

A scientific meeting dedicated to the world of single cell analysis

- 9:30-10:10 **Registration and Welcome breakfast**
- 10:10-10:30 **Prof. Barbara Montanini, Dr. Andrea Armirotti** *Welcome and introduction to the meeting*

Session Chair: Andrea Armirotti

- 10:30-11:00 **Erwin Schoof**, Technical University of Denmark; **Leveraging single cell proteomics to better understand human hematopoiesis**_virtual
- 11:00-11:20 **Francesca Ruscitti**, Chiesi Farmaceutici; **Multi-omics approach in support to target identification**_in presence
- 11:20-11:50 **Paul Humphrey and Christof Mitterer;** Thermo Fisher Scientific; **Single cell proteomics – Going beyond one cell at a time**_in presence
- 11:50-12:20 **David Hartlmayr,** Cellenion Proteomics Application Specialist**; An automated workflow for label-free and multiplexed Single Cell Proteomics using the cellenONE**_in presence
- 12:20-12:50 **Ronald Cutler,** Albert Einstein College of Medicine; **Exploring cell-to-cell variation during aging**_virtual

Young Session Chair: Lolita Piersimoni & Barbara Montanini

- 12:50-13:05 – Selected *Flash* oral communication from Poster session (3 young investigators, 5 mins)

- 13:05-14:15 **Lunch & Poster Session** *Towards Single Cells Proteomics*

Session Chair: Simone Sidoli

- 14:15-14:45 - **Pierre-Olivier Schmit**, Bruker**; Enabling routine single cell proteomics analysis: presenting the timsTOF SCP based solution**_in presence
- 14:45-15:15 – **Mario Armelao** – SCIEX; **Improved data-independent acquisition (DIA) and data-dependent acquisition (DDA) performance on low-level proteomic samples using a novel Zeno trap**_in presence
- 15:15-15:45 **Prof. Nikolai Slavov**, Director Single Cell Proteomics Center, Northeastern University, USA_ **Driving biology with single-cell proteomics: New data acquisition and interpretation methodology for sensitive protein analysis**_virtual
- 15:45-16:00 **coffee break**
- 16:00-16:30 **Claudia Ctortecka**, Broad Institute of MIT and Harvard; **Under the looking glass: revealing proteome heterogeneity at single-cell and low input using the timsTOF SCP technology**_virtual
- 16:30 – 17:00 **Karl Mechtler,** Research Institute of Molecular Pathology (IMP); **An automated workflow for multiplexed single-cell proteomics sample preparation**_in presence
- All speakers: Wrap-up/discussion/round table

This event is made possible thanks to the support of:

Thermo, Bruker, Euroclone/Cellenion, Sciex, Chiesi

Scientific & organizing committee:

Barbara Pioselli, Lolita Piersimoni, Silvia Catinella, Barbara Montanini, Andrea Armirotti , Simone Sidoli, Claudio Iacobucci