Precision proteomics for the characterization of follicular-patterned thyroid neoplasms entities

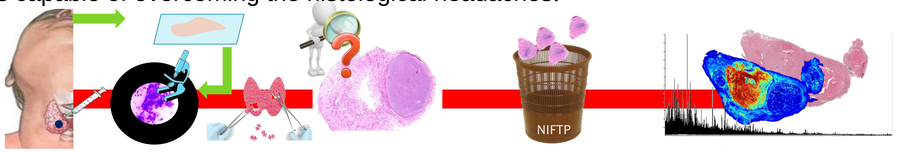
Isabella Piga[1]; Giulia Capitoli[2]; Francesca Clerici[1]; Vincenzo L'imperio[3]; Gabriele Casati[3]; Katherine A. Stumpo[4, 5]; Dale S. Cornett[4]; Andrew Smith[1]; Stefania Galimberti[2]; Fulvio Magni[1]; Fabio Pagni[3]

isabella.piga@unimib.it

[1]University of Milano-Bicocca, Department of Medicine and Surgery, Proteomics and Metabolomics Unit, Vedano al Lambro, Italy;
[2]University of Milano-Bicocca, Bicocca Bioinformatics Biostatistics and Bioimaging B4 Center, School of Medicine and Surgery, Vedano al Lambro, Italy;
[3]University of Milano-Bicocca, School of Medicine and Surgery, Pathology Unit, San Gerardo Hospital, ASST Monza, Monza, Italy;
[4]Bruker Scientific LLC, Billerica, MA;
[5]The Johns Hopkins University School of Medicine, Russell H. Morgan Department of Radiology and Radiological Science, Division of Cancer Imaging Research, Baltimore, MD

INTRODUCTION AND AIM

Noninvasive follicular thyroid neoplasms with papillary-like nuclear features (NIFTP) are low-risk thyroid lesions most often characterized by RAS-type mutations. However, the diagnostic category NIFTP is becoming a basket that includes not only classic tumors completely adherent to the original definition, but also a large number of questionable cases, due to pathologists current diagnostic limits. The current histological diagnostic criteria are still debated and even immunohistochemistry (IHC) and molecular approaches have not yet provided reliable diagnostic targets. The aim of this preliminary study is to characterize NIFTP lesions by Matrix-Assisted Laser Desorption/Ionization (MALDI)-Mass Spectrometry Imaging (MSI) in order to highlight proteomic signatures capable of overcoming the histological headaches.



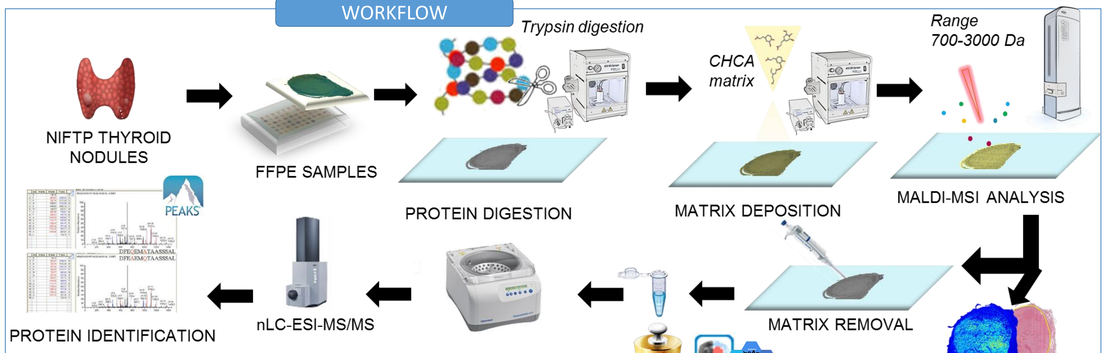
Cytological diagnosis
THY3 → Indeterminate

Indeterminate histology

✓ NIFTP RAS-mutated
✓ NIFTP RAS-wild-type

MALDI-MSI analysis

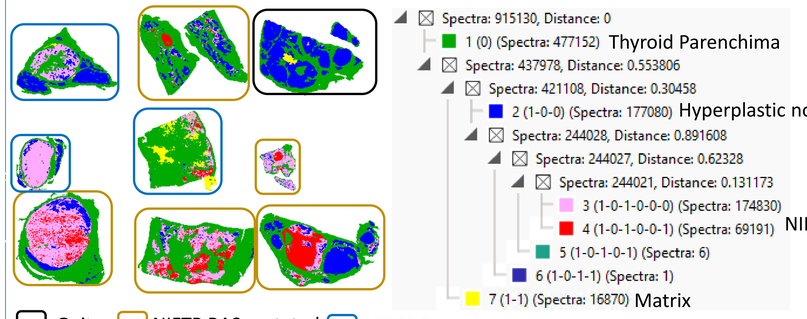
WORKFLOW



FFPE TISSUE SECTIONS (Spatial Resolution 50x50 µm)
5 NIFTP RAS-mutated, 3 NIFTP-RAS wild-type, 1 Goiter
TMA (Spatial Resolution 10x10 µm)
24 PTC, 8 FvPTC, 12 NIFTP, 4 Follicular adenoma, 2 Hurtle Adenoma

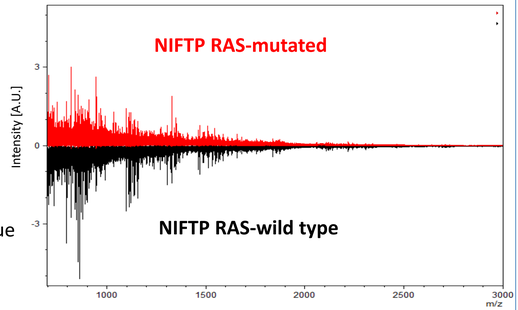
RESULTS

SEGMENTATION IMAGES: BISECTING K-MEANS – CORRELATION DISTANCE

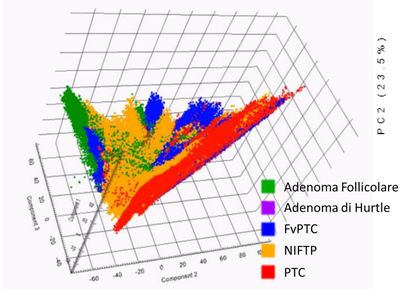


Goiter NIFTP RAS mutated NIFTP RAS wild type

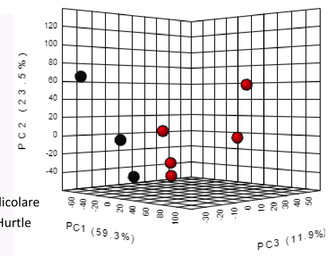
AVERAGE SPECTRA OF NIFTP ANNOTATED NODULES



PRINCIPAL COMPONENT ANALYSIS OF TMA DATA

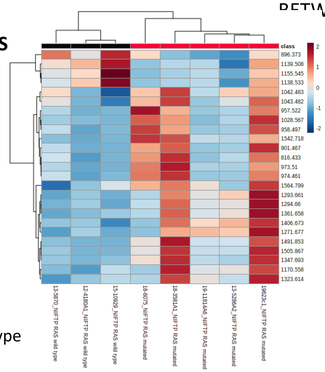


PRINCIPAL COMPONENT ANALYSIS



RAS-mutated RAS-wild type

HEATMAP



DIFFERENTIALLY EXPRESSSED SIGNALS BETWEEN NIFTP RAS-mutated AND NIFTP RAS wild type
ROC ANALYSIS: AUC ≥0.75 and p-value ≤0.05
3↑ 26↓

m/z ↑	Putative Identification	Error (ppm)
1491.8	H2B1B	19
1505.8	PPIA	74
1542.7	TPIS	-98
m/z ↓	**Putative Identification**	**Error (ppm)**
990.4	CO6A3	-17
950.5	RL13	-31
1155.5	MYH9	-98

These results underlined the unique capability of spatial proteomics to detect the proteomic signatures of RAS-mutated and RAS-wild-type NIFTP lesions highlighting proteomic alterations even within regions that are indistinguishable at the microscopic level, and the potential role of MALDI-MSI technology to support traditional pathology. Spatial-proteomics is an outstanding approach to differentiate NIFTPs from other follicular-patterned features and to characterize classic and atypical cases.

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