Poster number 718

BIOMARKER MONITORING BY QUANTITATIVE MALDI IMAGING: APPLICATION TO THE TRYPTOPHAN-KYNURENINE PATHWAY IN IMMUNO-ONCOLOGY

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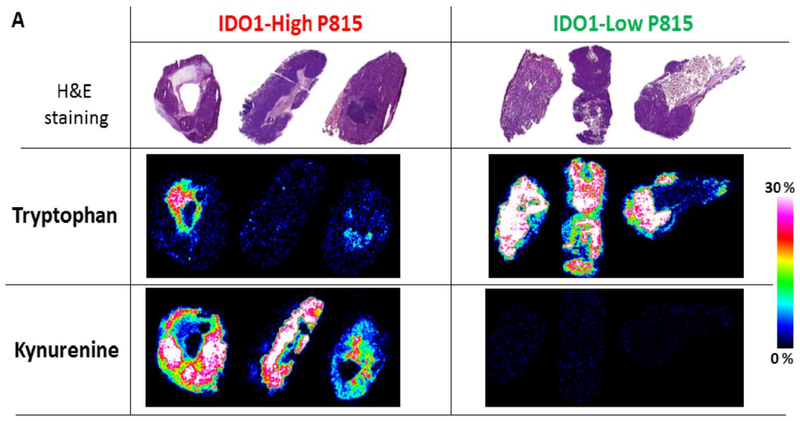
Summary

Inhibition of NK and effector T-cells functions, activation of regulatory cell populations are the main immunosuppressive effects of indoleamine-2,3-dioxygenase 1 (IDO1). By converting Tryptophan (Trp) into Kynurenine (Kyn), IDO1 is involved in the immune response homeostasis, and its dysregulated expression has been described in immune-related pathologies. Tumors also hijack this Trp-catabolizing enzyme to evade immune destruction. Thereby, IDO1 inhibitors are being developed to stimulate the anti-tumor immune responses. The existing and standard quantitation methods of IDO1 substrate and metabolite(s) are based on the total level of Trp and its metabolites determined by LC-MS/MS analysis in human plasma, cerebrospinal fluid and brain. Here we describe the detection, localization and absolute quantitation of Trp and Kyn by quantitative MSI in murine tumor models expressing various levels of IDO1. Using MALDI FTICR, we show the correlation between IDO1 expression and Trp to Kyn conversion. High definition images overlaying IDO1 and GCN2 immunostainings with Kyn metabolite molecular images underlined the tumor metabolism and heterogeneity. The development of immunotherapies such as IDO1 inhibitors requires a deep understanding of the immune system and cancer cells interplay and biomarkers characterization. Our data underlines that qMSI allows to study the spatial distribution and quantitation of endogenous immune metabolites for biology and pharmacology studies.

Material and Methods

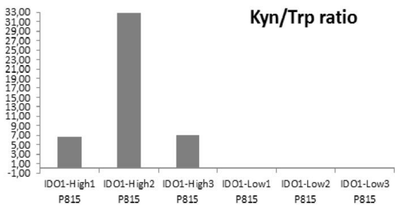
10µm thick tissue sections of P815 tumor xenografts (previously inoculated in BALB/c mice) were thaw mounted onto ITO coated slides. 2,5-dihydroxybenzoic acid (2,5-DHB) and 1,5-diaminonaphthalene (1,5-DAN) matrices were coated using TM-Sprayer (HTX) device. MSI analysis was performed using 7T MALDI FTI-ICR (SolariX XR, Bruker Daltonics,) in positive and negative ion modes (m/z range 50 – 1000) at high-resolution with an on-line calibration. Data acquisition, processing, and visualization were performed using the Flex software suite (ftmsControl 2.1.0, FlexImaging 4.1 and DataAnalysis 4.2). Quantification data analysis was done with Multimaging™ (ImaBiotech, France). This multimodal imaging platform combines Quantitative Mass Spectrometry Imaging (QMSI) and Microscopy Platform with Statistical Analyzes for the understanding the Omics information at cellular levels. Immunohistochemistry anti-IDO and anti-GCN2 (AbCam, UK) with rabbit polyclonal antibodies. Goat anti-rabbit IgG H&L (HRP) secondary antibody was used. Detection system was through horseradish peroxidase followed by Steady DAB/plus (brown chromogen).

Results



B

Average intensities	Kyn	Trp	Kyn/Trp ratio
IDO1-High1 P815	7,95E+05	1,19E+05	6,6751
IDO1-High2 P815	6,35E+05	1,93E+04	32,9253
IDO1-High3 P815	3,01E+05	4,24E+04	7,0913
IDO1-Low1 P815	9,89E+03	7,23E+05	0,0137
IDO1-Low2 P815	9,16E+03	4,90E+05	0,0187
IDO1-Low3 P815	9,02E+03	3,19E+05	0,0282



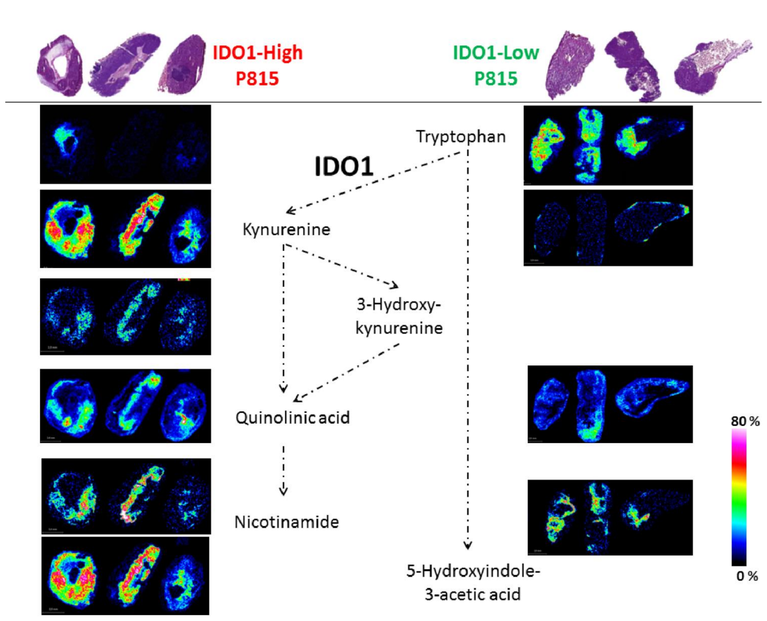
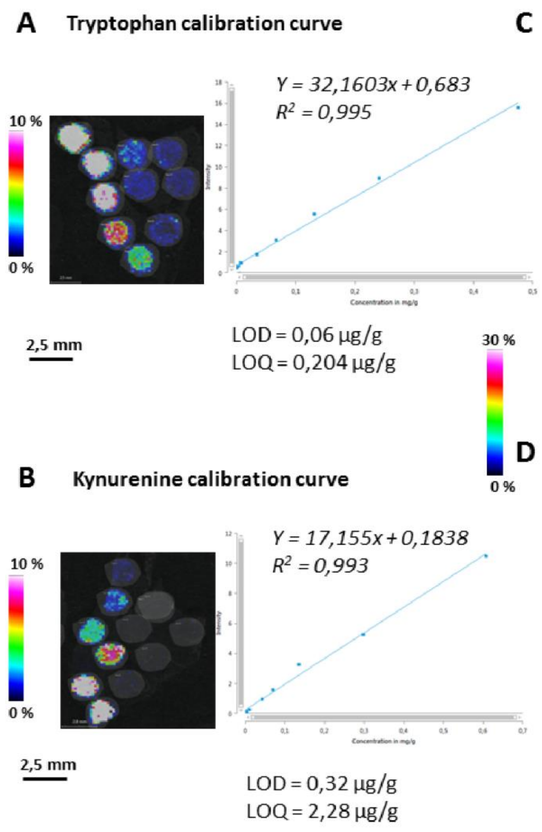


Figure 1: MALDI MSI of Tryptophan pathway: an insight in the tumor metabolism
Tryptophan and Kynurenine histological localization is shown in triplicates in both models (IDO1-High and -Low P815) (**A**). Average intensities of Trp and Kyn were then extracted from all ROIs and ratios (Kyn/Trp) calculated, raised and represented as a histogram for both mouse models (**B**). Trp catabolism pathway metabolites were detected using 7T-MALDI-FTICR MSI in biological triplicates of IDO1-High and -Low P815 (**C**).



C

Tryptophan	IDO1-Low P815		IDO1-High P815	
	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
Triplicate 1: 4,09 µg/g	2,83 µg/g	5,35 µg/g	BLOQ µg/g	BLOQ µg/g
Triplicate 2: 4,35 µg/g	4,65 µg/g	4,14 µg/g	BLOQ µg/g	BLOQ µg/g
Triplicate 3: 1,27 µg/g	0,86 µg/g	1,69 µg/g	BLOQ µg/g	BLOQ µg/g

D

Kynurenine	IDO1-Low P815		IDO1-High P815	
	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
Triplicate 1: 34,1 µg/g	BLOD µg/g	BLOD µg/g	36,08 µg/g	32,12 µg/g
Triplicate 2: 32,59 µg/g	BLOD µg/g	BLOD µg/g	33,26 µg/g	31,92 µg/g
Triplicate 3: 25,51 µg/g	BLOD µg/g	BLOD µg/g	27,80 µg/g	23,23 µg/g

Figure 2: Quantitative MALDI MSI of Tryptophan and Kynurenine
Calibration curves were performed using spiked analyte on 10 µm liver tissue sections. Internal standards mixed to the matrix were sprayed over all the ROIs. Multimaging™ software was then used to normalize and calculate the calibration curves equations, correlation coefficients and limit of detection (LOD) and quantitation (LOQ) (**A** and **B**). Absolute quantitation analysis on regions of interest (IDO1-Low and IDO1-High tumors) allowed to compare both models, each, in biological triplicates (triplicates 1, 2, 3) and technical duplicate (duplicates 1 and 2) (**C** and **D**).

Figure 3: High resolution imaging and definition: an access to the cell level
High definition scan of H&E-stained IDO1-High P815 tissue section was overlaid with Kyn molecular image (**A**). Trp and Kyn co-localization is shown in green and red colors, respectively (**B**). High resolution molecular Kyn image was overlaid with high definition GCN2 (**C**) and IDO1 (**D**) immune-stained images. Control immunostaining are shown in inserts. All these overlays were performed using ImaBiotech's multimodal platform: Multimaging™ software.

Figure 4: Tumor metabolic interactions with the microenvironment highlighted by MALDI MSI

Conclusion and Perspectives

Small molecule inhibitors of IDO1 are currently under preclinical and clinical development. We could position our quantitative MALDI MSI methodology at different levels during drug discovery, development and marketing:

i/ to monitor proximal **pharmacodynamics biomarkers** of drug efficacy, by measuring the early metabolic response (Trp and Kyn endogenous levels);
ii/ to study the histological **PK/PD relationship**, by running a **pharmacokinetic** study of the drug and showing the correlation between drug presence and target modulation in tumor microenvironment;
iii/ to further **characterize the mechanism of action** of the drug at the immune and metabolic levels;
iv/ to explore the putative **toxicity** of the drug;
v/ to mediate the relationship between inflammation, immune state and tumor diagnosis by highlighting indications for tryptophan degradation, measured by Kyn/Trp linked to other metabolites.