MALDI Imaging of an Anti-Cancer Drug in Tissue Samples of Mouse Mixoyd Liposarcoma

Introduction

Myxoid liposarcoma (MLS) is the second most common liposarcoma (35–40%). It arises mainly in the deep soft tissue of the extremities at a median age of incidence of 45 years. Histologically it is characterised by a myxoid stroma with a distinctive plexiform vascular network and low cellularity composed of round/oval non-lipogenic cells, lipoblasts and mature adipocytes. Generally, MLS has an indolent clinical progression, but a subset of patients shows a more aggressive behaviour.

MLS is more sensitive to chemo- and radio-therapy than other liposarcoma subtypes. The marine alkaloid trabectedin is very effective in this histotype, achieving a 90% overall control rate (complete response, partial response and stable disease) with a RECIST objective response rate of 50% and a progression-free survival of 17 months.

The anti-cancer drug of the thiazolidinedione class has been shown to be an effective treatment in various MLS PDX characterized by different sensitivity to trabectedin increasing its the efficacy. Drug distribution has a central role in the effectiveness of anticancer therapy and suboptimal drug concentration at the site of action can be an important factor in the ineffectiveness of chemotherapy and in the insurgence of resistance. In this field, mass spectrometry imaging has evolved as a valuable tool for the visualization of drugs in tumor sections.

Results

Sample Preparation

Several liver and MLS tumor tissue sections were mounted onto the stainless steel MALDI Target plate and then covered with HCCA (α-Cyano-4-hydroxycinnamic acid) MALDI matrix. A calibration curve was spotted on untreated sections. Figure 1 shows the spray result of the matrix application using a SunCollect pneumatic sprayer. The tumor tissues were homogeneously covered with HCCA.

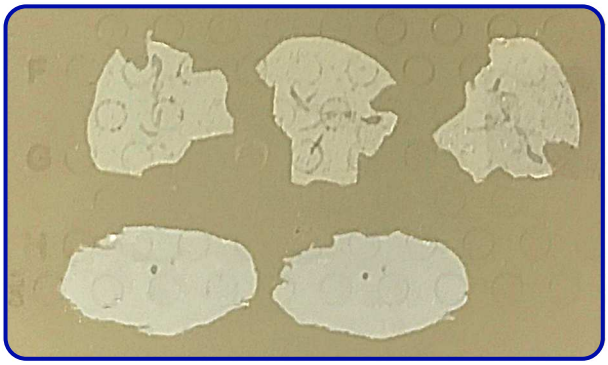

Figure 1: HCCA covered tissue sections of liver and MLS tumors mounted on a stainless steel MALDI target plate. The tissue section were covered with HCCA MALDI matrix using a SunChrom SunCollect pneumatic sprayer.

Mass spectrometry analysis

The drug spotted on tissue sections is clearly detectable as positive molecular ion (m/z 357.124) while it is absent in blank samples. The Q Exactive Orbitrap high resolution allows distinguishing easily the drug ion from interfering peak with similar molecular mass (Figure 2).

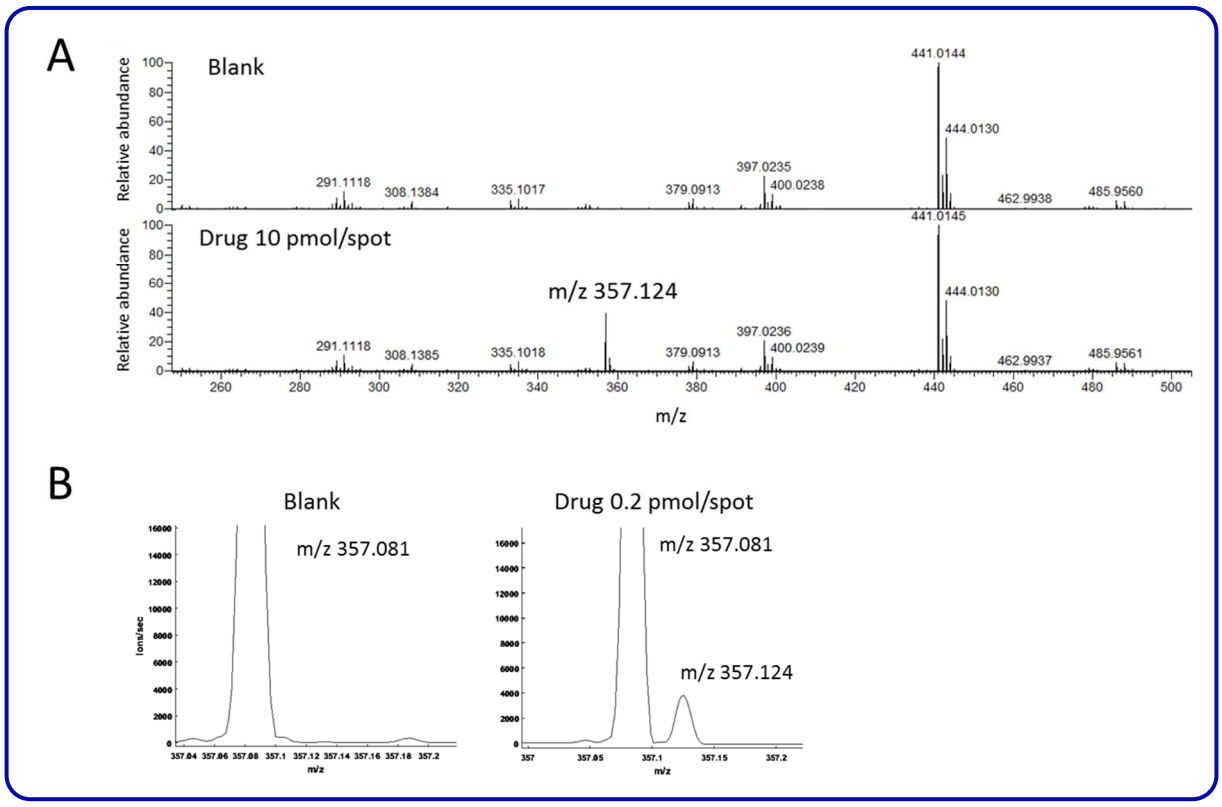

Figure 2: Representative mass spectra of untreated tissue sections and tissues spiked with the anti-tumor drug (A). Distinguishing drug ion from interfering peak in high-resolution MS (B).

MALDI Imaging

Spectra were recorded in positive ion mode on a Q Eaxctive Orbitrap Thermo Scientific equipped with the MassTech AP-MALDI UHR. The drug was spotted on untreated tissue section at increasing concentrations (0.1-2 pmol/spot) to build a calibration curve. The drug distribution was analyzed in tissue section cut from organs explanted 4h after oral treatment. Constant speed raster motion was

used with 100µm or 25µm spatial resolution and plate velocity dependent on scan time. Data was exported as an imzML file from Image quest (Thermo) and imported into MSiReader v1.00. The drug ion signal (tolerance 5ppm) was normalized in each pixel to the TIC.

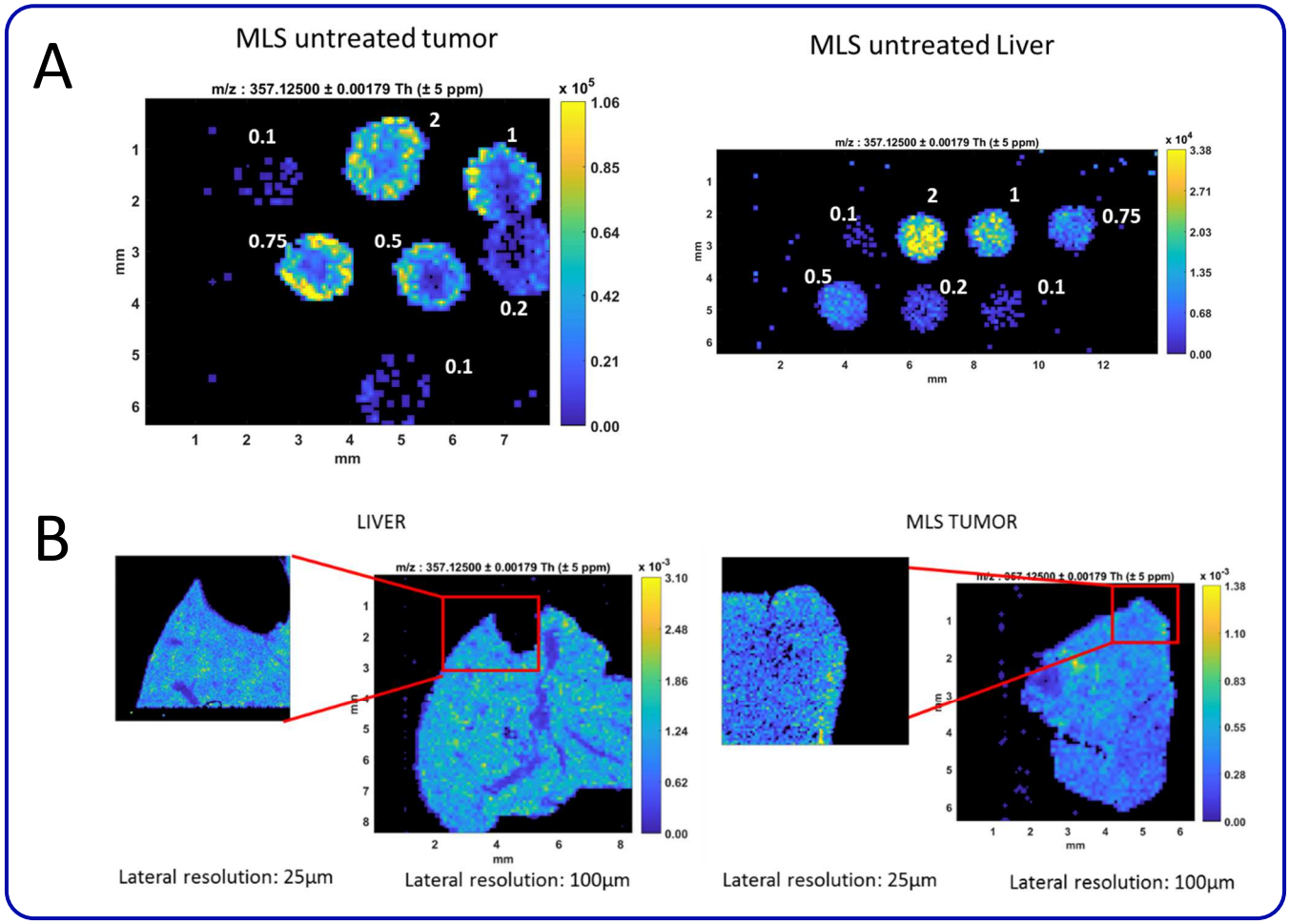

Figure 3: Detection of the anti-tumor drug spotted on untreated tissue sections at different concentrations (0.1 - 2 pmol/spot) (A) and MALDI-Imaging analysis of liver and tumor tissue sections (B).

The drug was clearly detectable with a limit of detection around 0.1pmol/spot and the ion signal increases linearly with the drug concentration (Figure 3). The distribution in tumor and liver section is quite homogeneous even if it is possible to identify area where the drug concentration appears lower. It is possible to visualize the distribution of the drug in tumors and livers imaged with pixel size of 25 µm (Figure 3).

Conclusion

This short study demonstrates the ability of detecting an anti-tumor drug in tissue sections and the visualisation of its spatial distribution by MALDI-MS-Imaging. The distribution of the drug was successfully visualised in tumor and liver section with an optimal limit of detection, great specificity and lateral resolution. The instrumental equipment used for the investigation enables scientists to gain knowledge about the distribution and metabolism of the drug in the organism, providing important information about effectivity and the biological role of the drug.

Experimental

Tissue Sample

Organism	Organ / Sample	Sample slide thickness	Sample origin
Mouse (*mus musculus*)	MLS / Liver tissue	10 µm	Fresh frozen

Instrumentation

Sample Preparation / Matrix Application	MALDI Imaging Analysis
SunChrom SunCollect	AP/MALDI (ng) UHR Ion-Source coupled to a Thermo Q Exactive Orbitrap

Matrix Application

Matrix solution	HCCA (α-Cyano) [10 mg/mL in Acetonitrile : H_2O (70:30)]				
Spray protocol	Z-Axis	No. of Layers	Flowrate	Spray Speed	Line distance
2.5 bars (36 PSI) Gas pressure	25 mm	12	Variable: (10; 20; 30; 40; 8x60 µL/min.)	600 mm/min.	2 mm

Ion Source Settings

Laser UHR attenuator	Column/Raw spacing	Pixel Duration	Velocity	Spray Voltage	Capillary Temp.	S-lens RF level
25%	100µm	0.27 sec	21.5 mm/min	3 kV	250°C	80
25%	25 µm	0.27 sec	5.4 mm/min	3 kV	250°C	80

Mass Spectrometer Settings

Polarity	Scan Type	Mass Range	Resolution	MicroScan	AGC target	Max Injection Time
Positive	Full Scan	100-1000	35000	1	$5e^6$	100ms