MULTIMODAL IMAGING OF LIPIDS IN A ZEBRAFISH MELANOMA MODEL BY PET AND DESI-MS

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INTRODUCTION

Since the first visualization of a tumour over a 100 years ago, imaging has become an essential tool in cancer research. Techniques, such as PET, have enabled the detection of small tumours and metastases in 3D. However this targeted technique requires the use of PET tracers which are primarily radiolabelled chemical compounds. Mass spectrometry imaging (MSI) is an untargeted technique providing a wide range of molecular information that can be imaged without the need for a radiolabelled tracer.

In this work we compare and contrast the use of a radioactive FTHA[1] tracer administrated to a tumour-bearing zebrafish and imaged by PET, with imaging cold FTHA by DESI mass spectrometry.

METHODS

Zebrafish sample preparation

The lipoprotein lipase mutant and wild type zebrafish used for the PET experiment were immersed for 30 min in 100 mL of water containing 60MBq (~ 0.5µg) of [^{18}F]fluoro-6-thia-heptadecanoic acid (FTHA) tracer (figure 1).
Wild type zebrafish used for the DESI imaging experiment was immersed for 30 min in 100 mL water containing 0.5 mg of [^{19}F]fluoro-6-thia-heptadecanoic acid (FTHA) tracer. Fish were then placed in cuvettes (held in place with sponge) and cuvettes placed in falcon tubes filled with ice to maintain anesthesia.

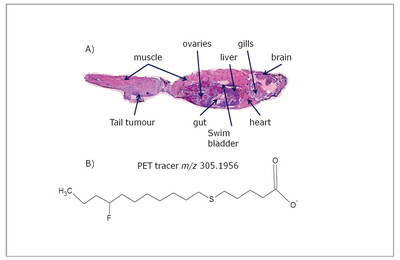

Figure 1. A) H&E stain image of a lipoprotein lipase mutant zebrafish. B) Chemical formula of the [^{18}F]fluoro-6-thia-heptadecanoic acid (FTHA) tracer.

PET

Static PET scans were then performed for 5 and 10 minutes on a pre-clinical PET scanner (Inveon, Siemens, Germany).

Tissue sections

The tumor bearing zebrafish was culled immediately after PET scanning, and flash-frozen in isopentane.
Sections of both zebrafish were produced using a cryotome at 12 µm onto standard glass slides.

Mass spectrometry

MSI experiments were carried out using a modified 2D DESI stage (Prosolia, US) mounted on a Waters Xevo-G2-XS mass spectrometer (figure 2).
DESI spray conditions were set at 2 µl/min, 95:5 methanol:water with a nebulising gas pressure of 4 bar.

Instrument settings

Polarity:	Negative and Positive
Mass range:	50 -1,200 *m/z*
Pixel size:	100 µm

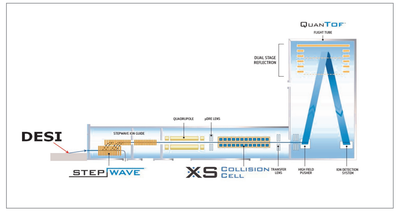

Figure 2. Schematic of the DESI Xevo G2-XS mass spectrometer.

Data management

PET data were analyzed and visualized using the Inveon Research Workplace.
MSI data acquired were processed and visualized using High Definition Imaging 1.4 (HDI) software for detailed image analysis. All ion images were TIC normalized.

RESULTS

Multimodal imaging with the tracer

PET experiments were carried out using [^{18}F] FTHA, which is a palmitic acid analogue used to visualise lipid metabolism in tumours. As seen in figure 3,A) the PET scan of the wild type zebrafish shows that FTHA tracer was up taken mainly in the gut part of the animal, with some also in the tail and the head.

The uptake of the tracer in the tumour bearing zebrafish shows a similar up take with some in the tumour region of the animal, however less than expected. Furthermore the uptake of the tracer within the tumour looks homogenous within the spatial resolution of the PET scan.

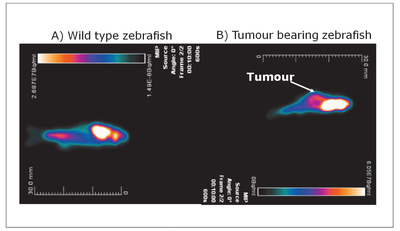

Figure 3: PET images of [^{18}F] FTHA tracer in the Inveon Research Workplace software.

With DESI imaging, the FTHA tracer was only observed within the zebrafish where the concentration was approximately 1,000 higher. [^{19}F]FTHA tracer was also localised in the wild type within the gut, tail and head of the animal (see figure 4). However the [^{18}F]FTHA tracer was not detected in the tumour bearing zebrafish, presumably due to lack of sensitivity.

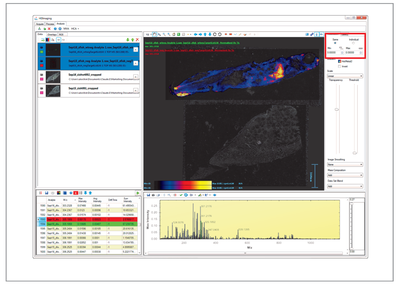

Figure 4: TIC normalized ion images of FTHA tracer in HDI 1.4, displayed with the same intensity scale, overlaid with the photographic images taken prior to DESI MSI acquisition.

Metabolites and lipids in the tumor region

DESI-MS offers a complementary imaging technique which allows the visualization of both tracer and endogenous lipid profiles. Within the acquired MS data it was possible to produce mass spectra containing rich molecular ion information from both lipids and metabolites.
Zebrafish tissue sections were first analyzed in negative mode, followed by the analysis in positive ion mode.

Using the "Clone ROI" functionality in HDI 1.4, ROIs containing the same number of pixels were drawn on the tumour region and the non-tumour tissue of both zebrafish. Continuum combined MS spectra for each ROI were generated and contrasted for the positive and negative DESI imaging datasets.

In figure 6,A), it can be observed that the lipid signal is enhanced on average by a factor 3-4 in the tumour region.
In figure 7,A) it can be observed that the lipid signal is significantly enhanced in the tumour region compared to the non-tumour region of the same zebrafish.

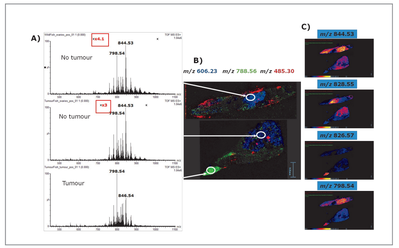

Figure 6: Positive mode A) MS spectra from ROIs on non tumour and tumour regions, B) RGB overlay , C) example of TIC normalized ion images from lipid species displayed with the same intensity scale.

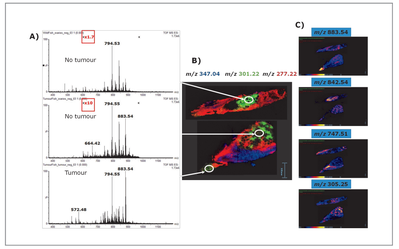

Figure 7: Negative mode A) MS spectra from ROIs on non tumour and tumour regions, B) RGB overlay , C) example of TIC normalized ion images from lipid species displayed with the same intensity scale.

In addition, a few endogenous species were mainly, or exclusively, present in the tumor region as seen in figure 8, in both positive and negative mode.
Figure 8 displays the TIC normalized ion images of m/z 766.5 and 788.5 in positive mode of the wild type and tumour bearing zebrafish. These are displayed on the same intensity scale. Also from the negative ion mode, m/z 526.3, 572.5, 720.5 and 734.5 are displayed.

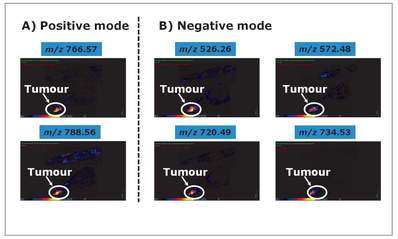

Figure 8: Ion images of endogenous species that are highly abundant in the tumour region of the zebrafish, A) positive ion mode, B) negative ion mode.

Further experiments were carried out on a tumour bearing serial section by DESI-MS/MS to identify some of the tumour specific molecules displayed in figure 8.
A low energy CID tandem mass spectrum was acquired from m/z 766.57 using argon collision gas.

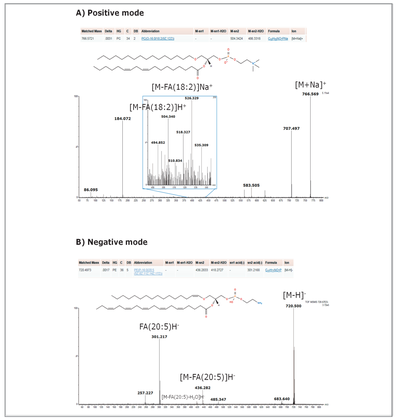

Figure 9: A) MS/MS spectrum of m/z 766.56 acquired directly from the tumour region by DESI and was tentatively identified as PC(O-16:0/16:2) Na^+ . B) MS/MS spectrum of m/z 720.5 acquired directly from the tumour region by DESI and was tentatively identified as PE(P-16:0/20:5) H^- .

Figure 9. A displays the MS/MS spectrum of m/z 766.56. A tentative identification was made using the Lipid Maps database (http://www.lipidmaps.org/tools/ms/) as a PC(O-16:0/16:2) Na^+ where the fatty acid fragment M-Sn1 m/z 504.34 was detected along with M-Sn1+Na+ m/z 526.33.

In negative mode, lipid m/z 720.49 was identified as PE (P-16:0/20:5)H^- with the presence of a fragment FA(20:5)H^- at m/z 301.21 and the loss of the fatty acid chain fragment at m/z 436.28 (figure 9,B).

Tumour heterogeneity by DESI imaging MS

Tumour heterogeneity is a major problem for diagnosis and therapeutics. Metabolic processes, including lipid biosynthesis, can be enhanced as part of cancer perturbations at the metabolic level. With their critical role in membrane formation and signalling, lipids can be significantly altered during cancer progression. With PET images, the distribution of the PET tracer was homogeneous, whereas in the DESI ion images significant heterogeneity was seen.
Figure 10 demonstrates examples of abundant lipids localizing differentially within the tumour region, in both positive and negative mode. This tumour heterogeneity was not observed within the PET image where the tracer was distributed homogenously within the tumour, primarily due to the spatial resolution of PET within this small tumour.

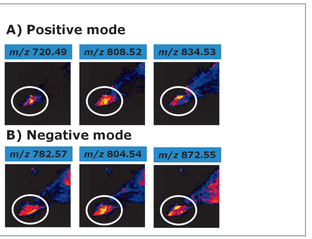

Figure 10: Ion images of endogenous species showing different distribution of lipids within the tumour region of the zebrafish, A) in positive mode, B) in negative mode.

CONCLUSION

- **PET and DESI imaging techniques produced complementary information.**
- **PET imaging showed the *in-vivo* homogenous uptake of the FTHA tracer into the tumour region of the lipoprotein lipase mutant zebrafish due to poor spatial resolution**
- **By DESI imaging, the tracer was only observed in the wild type zebrafish ([^{19}F]FTHA tracer) and not in the tumour bearing zebrafish ([^{18}F]FTHA tracer) .**
- **DESI imaging illustrated the lipid heterogeneity within the tumour due to the untargeted experiment and the higher spatial resolution.**

References

1. Stocklin et a;. The Journal of Nuclear Medicine, 1991, Vol. 32, No. 10 1888-1896

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