Comparative Analysis of Tumours, Their Metastases and Derived Primary Cell Lines by Rapid Evaporative and Desorption Electrospray Ionization Mass Spectrometry

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INTRODUCTION

Breast cancer is the most common malignancy in women. Almost a third (30%) of the patients with early-stage breast cancer will experience distant **metastases**, which together with cancer recurrence accounts for 90% of the total breast cancer mortality, thus metastases remain the principal cause of cancer death and *is one of the greatest challenges both cancer researchers and oncologists encounter*.

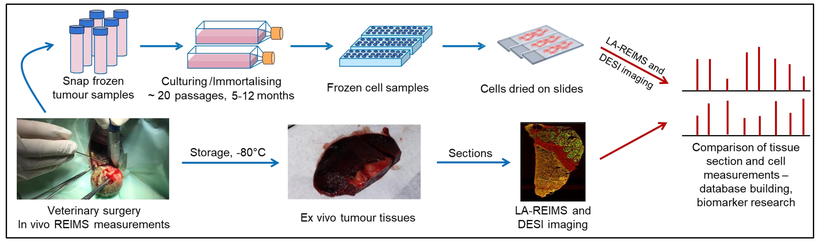
As part of the treatment, in some cases, it is the **primary tumour** that can be removed, and the **metastasis** cannot or not entirely, while in other cases, it is the other way around. In case, both are removed, their mutual investigation provides an opportunity to explore the links between the two. Although the primary tumour and metastasis cells are the same type of origin, it is a question to what extend their chemical fingerprints resemble, **what information their fingerprints give of both vice versa and what information their cell lines themselves give about them**. With a better understanding of the tumours' characteristics, therapies might be developed on their gained cell lines. In this study, we examined the very basics of this proposition.

AIMS

Investigate the relationships between the tumour, its metastasis and their primary cell lines using chemical imaging, Laser-Assisted Rapid Evaporative Ionization Mass Spectrometry (LA-REIMS) and Desorption Electrospray Ionization (DESI)

1) Understand the characteristics of the original tumours & the primary tumour and metastasis relationships better
2) Examine the results of the cell measurements obtained by the two technologies
3) Compare the tumours and their cell lines, explore their sensitivity profiles, identify characteristic peaks and make an attempt to look for biomarkers
4) Make a benchmark study comparing LA-REIMS to DESI

EXPERIMENTAL WORKFLOW



1) Spontaneous tumours were sectioned and characterized using Optical Parametric Oscillator (OPO) Laser-Assisted REIMS coupled to an imaging platform and Desorption Electrospray Ionization with 50 µm resolution
2) Immortalized cell lines were established from the tumours removed during veterinary surgeries, and the cell samples taken during culturing were dried on slides and later measured by the imaging platforms
3) The tissue section and cell measurement results were compared to investigate the possibility of database building for the use of iKnife and to examine the relevance of further dietary experiments on cells
4) Compare the results of the two technologies and examine their feasibility for the tumour and cell measurements

Measurements were carried out by a Xevo™ G2-XS TOF MS (Waters Corporation) equipped with a corresponding DESI and REIMS™ source, data was acquired in negative ion mode in 50-1200 *m/z* range

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RESULTS

1. Tissue measurements by DESI and LA-REIMS imaging

Two tumour – tumour metastasis pairs were examined: a breast adenocarcinoma (BA) and its lung metastasis (LM) and a breast simplex tubulopapillar carcinoma (BSTC) and its skin metastasis (SM)

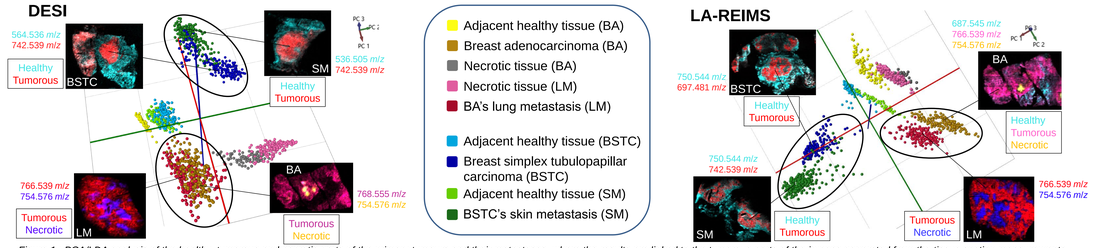

Figure 1.: PCA/LDA analysis of the healthy, tumorous and necrotic parts of the primary tumours and their metastases, where the results are linked to the tumorous parts of the images generated from the tissue section measurements

Great correlations between the primary tumour and its metastasis
(PCA space distance 0.6-0.9 (DESI) and 1.0-1.5 (LA-REIMS))

- Strong similarities among the healthy and necrotic parts of the tissues (PCA space distance 0.6-1.1 (DESI) and 1.0-1.5 (LA-REIMS))
- Significant differences among the three distinguished parts of the tissues (PCA space distance 1.5< (DESI) and 2.2< (LA-REIMS))

According to the cross-validation results: the original tumour is identifiable based on the patterns detected in the respective metastasis

Similar results obtained by both DESI and LA-REIMS technologies
LA-REIMS imaging is suitable for distinguishing the tumorous, healthy and necrotic parts of the tissue sections just like a commercially available technique

2. Cell measurements

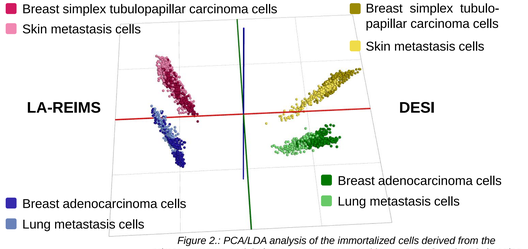

Figure 2.: PCA/LDA analysis of the immortalized cells derived from the primary tumours and their metastases measured by LA-REIMS and DESI imaging

Cells are clearly distinguishable by their origin and measurement method

3. Tumour tissue and cell comparability

Cell cultures and the tumorous parts of tissues were compared to get a comprehensive overview about the similarities and differences between them

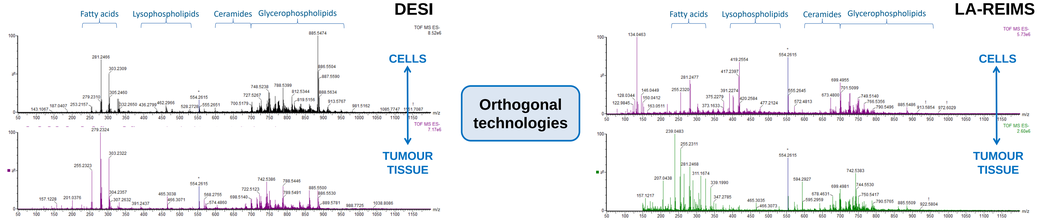

Figure 3.: Comparison of the immortalized homogenous cells and their respective heterogenous tumour tissue sections in case of the breast simplex tubulopapillar carcinoma measured by DESI and LA-REIMS imaging

Despite differences between cells and tumours, similarities in the most important species (separating tumour from healthy) justify further dietary experiments

4. Tumour tissue comparison by DESI and LA-REIMS imaging

The most abundant ions obtained from the measurements were examined, similarities and differences between the two technologies were compared

DESI imaging – BA tissue (tumorous part)

m/z	Molecule(s)	Adduct
722.513	PE(P-36:4)	[M-H]⁻
748.529	PE(P-38:5)	[M-H]⁻
750.544	PE(P-38:4)	[M-H]⁻
766.539	PE(38:4)	[M-H]⁻
778.576	PE(P-40:4)	[M-H]⁻
788.545	PS(36:1)	[M-H]⁻
810.529	PS(38:4)	[M-H]⁻
812.545	PS(38:3)	[M-H]⁻
838.560	PS(40:4)	[M-H]⁻
857.519	PI(36:4)	[M-H]⁻
862.560	PS(42:6)	[M-H]⁻
885.550	PI(38:4)	[M-H]⁻

LA-REIMS imaging – BA tissue (tumorous part)

m/z	Molecule(s)	Adduct
642.487	CerP(36:2)	[M-H]⁻
678.463	CerP(36:2)	[M+Cl]⁻
687.545	CerPE(36:1)	[M-H]⁻
699.497	PA(36:2)	[M-H]⁻
	PE(34:1)	[M-NH$_3$H]⁻
722.513	PE(P-36:4)	[M-H]⁻
744.555	PE(36:1)	[M-H]⁻
748.529	PE(P-38:5)	[M-H]⁻
750.544	PE(P-38:4)	[M-H]⁻
752.560	PE(P-38:3)	[M-H]⁻
766.539	PE(38:4)	[M-H]⁻
778.576	PE(P-40:4)	[M-H]⁻
794.570	PE(40:4)	[M-H]⁻
885.550	PI(38:4)	[M-H]⁻

Table 1-2.: The most abundant ions measured by DESI and LA-REIMS imaging in the tumorous part of the breast adenocarcinoma tissue, showing the major similarities and differences between the two technologies referring to the lower mass spectra in Figure 3.

Mainly phosphatic acids (PA), phosphatidylethanolamines (PE) and phosphatidylserines (PS) were identified, besides some ceramides and some characteristic phosphatidylinositols (PI)

DESI and LA-REIMS imaging – BA cells

m/z	Molecule(s)	Adduct	
671.466	PA(34:2)	[M-H]⁻	More abundant in LA-REIMS spectra
673.481	PA(34:1)	[M-H]⁻	
699.497	PA(36:2)	[M-H]⁻	
	PE(34:1)	[M-NH$_3$H]⁻	
701.513	PA(36:1)	[M-H]⁻	
	PE(34:0)	[M-NH$_3$H]⁻	
725.513	PA(38:3)	[M-H]⁻	
	PE(36:2)	[M-NH$_3$H]⁻	
727.529	PA(38:2)	[M-H]⁻	
	PE(36:1)	[M-NH$_3$H]⁻	
735.474	PA(36:2)	[M+Cl]⁻	
744.555	PE(36:1)	[M-H]⁻	
788.545	PS(36:1)	[M-H]⁻	More abundant in DESI spectra
819.518	PG(40:7), PS(40:5)	[M-H]⁻	
861.550	PI(36:2)	[M-H]⁻	
885.550	PI(38:4)	[M-H]⁻	
887.563	PI(38:3)	[M-H]⁻	
889.581	PI(38:2)	[M-H]⁻	

Table 3.: The major differences between the breast adenocarcinoma cells measured by DESI and LA-REIMS imaging (Figure 2.)

CONCLUSION

- **Primary tumours can be identified based on the chemical fingerprints of the metastases, both for tissue sections and cells**
- **There are major differences between cell and tumour tissue chemical fingerprints, but the most important species separating tumour from healthy are similar indicating the relevance of further dietary experiments on cells**
- **LA-REIMS and DESI imaging provided rich molecular profiles of cell lines and tumour sections, and their combined use provides comprehensive information as they are orthogonal technologies**
- *Future plans: Primary tumour and metastasis cells showed high degree of similarity, with the establishment of one of these cell lines, cells could be subjected to different dietary experiments and a successfully developed diet could be examined in vivo in an animal model for tumour suppression*