Micro-elemental mapping of trace elements in breast tissue

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Introduction:

The aim of this experiment is to map the distribution of Zn, Fe, Cu and Ca in breast cancer tissue and to compare their levels with surrounding normal tissue. Identifying the relative differences of trace elements levels at the cellular level between the normal and the cancerous tissue may clarify how the distribution of elements is related to progress of the disease. Additionally, the results obtained would be helpful for other research groups who are interested in developing therapeutic strategies for breast cancer. Oestrogen is a primary female sex hormone that acts by binding and activating protein molecules known Oestrogen Receptors (ER). Oestrogen is important for the normal growth and the development of the mammary gland. Breast cancer progression is influenced by oestrogen. The distributions of the Zn, Fe, Cu and Ca elements at the cellular resolution were correlated with the oestrogen receptor status. In this study, 16 positive oestrogen receptor (ER+) sample and 12 negative oestrogen receptor (ER-) samples were investigated.

Method:

The data was collected at Hasylab, beamline L (Hamburg, Germany). At the energy of 11 keV used in this study an on sample spot size of approximately 15µm x 15µm was obtained. A stepwise scan was used with 5-seconds measurement time at each point. The fluorescence signal is recorded using a Peltier cooled energy dispersive Si drift detector (Radiant, Vortex). The samples are formalin fixed paraffin embedded tissue of human primary invasive breast cancer. The samples were in the form of tissue micro arrays consisting of 1.0 mm diameter sections of tissue. Two slices were cut from the paraffin block, one being 10µm thick the other being 5µm thick and cut adjacent to the 10µm slice. The 10µm thick slice was mounted on a 4µm ultralene XRF film. The 5µm thick slice was mounted on a standard glass slide and then stained using Hematoxylin and Eosin (H & E) stain. This slide was then optically imaged to produce high resolution images which clearly identify tumour regions in the samples and can be used as a reference slide for the elemental distribution maps produced from the experimental slide.

Results

As an example, figure (1) below shows a reference slide, and elemental maps of Zn, Fe, Ca and Cu distribution in an ER (-) sample. The reference slide is stained with a haematoxylin dye which stains the nuclei structures with a purple-blue colour. The ROI is shown at a higher magnification to display the details of this region.

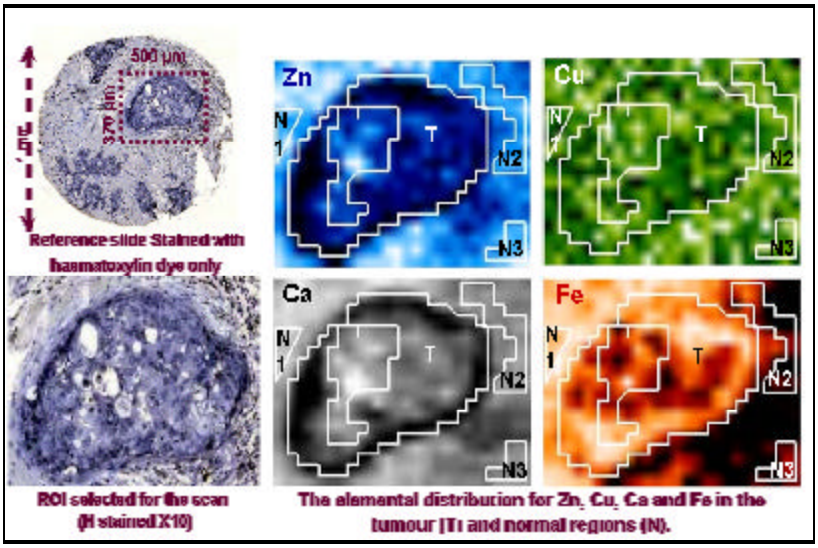

Figure 1: A stained reference image of breast sample. The dark areas are the cancer cell regions. The corresponding Zn, Fe, Cu and Ca distribution maps in the tumour and the normal regions.

An overview of the statistical analysis and the percentage difference of elemental mean levels in the tumour breast tissue compared to the normal

tissues are listed in table (1). The higher elements concentrations for Zn, Cu and Ca are strongly correlated with areas of tumour regions with an increase of 69.24%, 14.87 % and 28.08% respectively compared to the normal areas within the sample. However, the distribution of Fe does not follow the same pattern of the other elements inthe tumour and the normal regions.

Sample: A3						ER (-)					
Zn						Cu					
	N1	N2	N3	Average (N)	T		N1	N2	N3	Average (N)	T
Mean	7.12	8.33	7.72	7.72	13.06	Mean	1.15	0.92	0.99	1.02	1.13
Min	5.27	6.24	5.58	5.70	6.32	Min	0.51	0.38	0.47	0.46	0.21
Max	8.39	10.42	10.96	9.92	19.45	Max	1.70	1.52	1.67	1.63	2.10
SD	0.92	0.94	1.37	1.08	2.65	SD	0.35	0.31	0.37	0.34	0.39
Percentage Difference: 69.24%						Percentage Difference: 14.87%					
Ca						Fe					
	N1	N2	N3	Average (N)	T		N1	N2	N3	Average (N)	T
Mean	63.67	81.85	87.51	77.68	104.84	Mean	4.92	22.33	23.12	16.79	10.34
Min	55.00	67.12	74.57	65.56	46.01	Min	2.51	8.02	13.37	7.96	3.51
Max	73.02	99.34	113.45	95.27	166.66	Max	6.52	40	40	28.84	36.08
SD	5.50	7.45	12.28	8.41		SD	1.10	10.18	9.39	6.89	5.16
Percentage Difference: 28.08%						Percentage Difference: - 53.67%					

Table 1: Overview of statistical analysis and percentage difference between the mean levels of elements. A negative value represents a decrease in the tumour and a positive value an increase.

From the data analysed to date, we have found a significant difference in the percentage increase of Zn, Cu and Ca mean levels in tumour breast tissue compared to normal tissues between ER(+) and ER(-) breast tissue cancers ($p<0.05$). Table (2) show an overview of the statistical analysis.

	Sample	ER+	%diff of the mean			
			Ca	Fe	Cu	Zn
Average of %diff of the mean for ER (+)			75.65138	11.12232	40.74108	116.8979
	Sample	ER-	%diff of the mean			
			Ca	Fe	Cu	Zn
Average of %diff of the mean for ER (-)			40.21034	-8.58732	10.99068	60.5846
P-Value			<0.05	0.201103	<0.05	<0.05

Table 2: show an overview of the statistical analysis and percentage difference of Zn, Cu, Ca and Fe mean levels in tumour breast tissue compared to normal tissues between ER(+) and ER(-) breast tissue cancers.

Conclusion:

This work will enable us to further understand the distribution of iron, copper and zinc in invasive ductal carcinoma of breast. It will add to our existing data base and hence help us to correlate the Fe, Cu, Zn and Ca levels with other markers particularly the oestrogen receptor status of the patient. We will also be looking at correlations with other markers. We need to collect more data in order to obtain equal amounts of ER (+) and ER (-) samples.