

Micro-elemental mapping of Zn in breast tissue

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Introduction:

Excessive accumulation of Cu, Zn and Fe in breast cancer has been reported in a number of our studies (Geraki *et al* (2002, 2004), Farquharson *et al* (2007, 2008, and 2009)). Our previous work at beamline L investigated the zinc levels in human primary tumour tissue compared to surrounding normal tissue in samples of both oestrogen receptor positive (ER+ve) and ER-ve invasive ductal carcinoma of breast (Farquharson *et al* 2009). The study has shown that the mean total Zn content for each sample (i.e. tumour and surrounding regions) was approximately 60% higher in the ER+ve samples than that in the ER-ve samples. A subgroup of ER+ve breast cancer patients has been selected to be treated with tamoxifen. The aim of this experiment is to map the distribution of Zn in breast cancer tissue and to compare their levels with surrounding normal tissue and correlated this with the progress of the disease among this group of patients. In this study a total of 152 tumours and 99 normal breast ROIs were scanned and analyzed. The results obtained were used to identify the relative differences of Zn levels at the cellular level between relapsed and non relapsed status, and the survival status of these patients.

Method:

The data were collected at Hasylab, beamline L (Hamburg, Germany). At the energy of 11 keV used in this study an on sample spot size of approximately 15 μ m x 15 μ m was obtained. The fluorescence signal is recorded using two Peltier cooled energy dispersive Si drift detectors (Radiant, Vortex). A stepwise scan was used, and utilising the two detectors to collect the fluorescence response enabled a 3s measuring time at each point. The samples are formalin fixed paraffin embedded tissue of ER+ve human primary invasive breast cancer. The samples were in the form of tissue micro arrays consisting of 1.0 mm diameter sections of tissue. For more information about sample preparation see Farquharson *et al* 2009.

Results

As an example, figure (1) below shows a H & E stained reference slide, and elemental maps of Zn, distribution in an ER (+) sample.

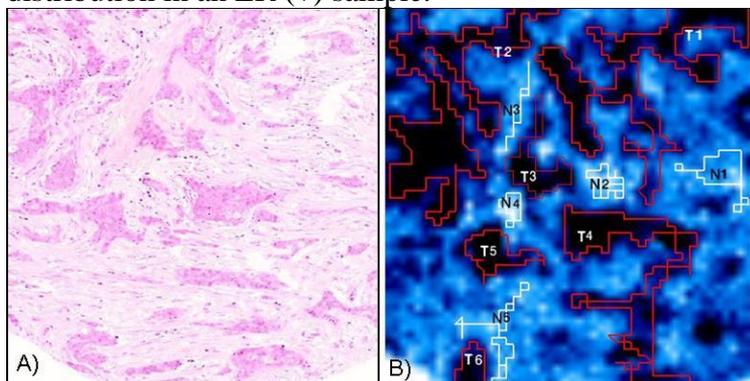


Figure 1: A) A stained reference image of breast sample. The dark areas are the cancer cell regions. B) The corresponding Zn, distribution maps in the tumour and the normal regions.

Results:

An overview of the statistical analysis and the percentage difference of Zn mean levels in the tumour breast tissue compared to the normal tissues is listed in table (1). The Zn levels were higher in the tumour areas compared to the normal areas with the mean levels in the tumour levels being approximately 95% higher than the mean normal levels.

Group	N	Zn-Mean	% difference	Std. Deviation	P value -Mann Whitney
Tumour	152	.12359446	94.7	.032	<0.001*
Normal	99	.06347841		.023	

Table 1: Overview of statistical analysis and percentage difference between the mean levels of Zn. * Significant difference at 99 % confidence level.

When comparing the Zn levels between the relapsed tumour and non relapsed tumour groups it was found that the mean total Zn was approximately 24% higher in the tumour relapsed regions with $P < 0.001$. The mean levels of Zn in the tumour regions of deceased subjects did not differ significantly from that in the tumour group of surviving subjects. The average Zn level was elevated in the relapsed normal group compared to the non relapsed normal group, but that elevation was not significant. In the normal deceased group and surviving group ROIs, the differences in Zn levels have been found to be not significant. Table (2) show an overview of the statistical analysis for these groups.

	Group	N	Zn-Mean	% difference	Std. Deviation	P value -Mann Whitney
Tumour	Non Relapsed	102	.1144	24.18	.030	<0.001*
	Relapsed	50	.1421		.027	
Tumour	Surviving	105	.1237	-0.37	.030	0.578
	Deceased	47	.1232		.03	
Normal	Non Relapsed	65	.0610	11.76	.017	0.982
	Relapsed	34	.0681		.0317	
Normal	Surviving	76	.0598	-40.59	.017	0.375
	Deceased	23	.0355		.07	

Table 1: Overview of statistical analysis and percentage difference between the mean levels of Zn in relapsed and non relapsed status, and deceased and survival status of the patients. Negative value represents a decrease. The percentage difference calculated with respect to non-relapsed and survival groups respectively. * Significant difference at 99 % confidence level

Conclusion:

Basically, there is significantly more Zn in the patients that relapsed than those that did not relapse. This work will enable us to correlate the Zn levels in ER (+) invasive ductal carcinoma of breast with the status of the patient after being treated with tamoxifen. The results can be used to help in evaluating the effectiveness of using tamoxifen for treatment. We need to scan more samples in order to obtain significant data.

References

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