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The localisation and micro-mapping of copper and other trace elements in breast tumours using a synchrotron micro-XRF system

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Abstract

Trace elements have critical roles in cancer biology. The quantity and distribution of the elements Cl, Ca, K, P, S, Ti, Fe, Cu and Zn in samples of primary breast cancer have been assessed. The samples were formalin fixed tissue specimens formatted as microarrays of cores 1.0 mm diameter and 10 μm thick each. The data were obtained using a synchrotron X-ray fluorescence microprobe system. The spatial resolution of elemental maps was approximately 20 μm. Maps were compared with light transmission images of the samples and then the images were stained for cancer. The synchrotron system proved successful in producing data that could be mapped into high-resolution images where clear structure could be identified. Correlation of these distributions with the concentrations of cancer cells was achieved in some samples.

Keywords: X-ray fluorescence (XRF); Synchrotron radiation; Micro-XRF; Breast cancer; Trace elements

1. Introduction

For over 30 years an active area of research has been the study of concentrations of trace elements in relation to breast disease, in order to help understand the disease process. Garg et al. (1994) and Ng et al. (1997) utilised neutron activation analysis (NAA) to study paired breast tissue specimens (healthy and tumours), while the same technique was used by Kanias et al. (1994) to compare samples of fibrocystic disease and fibroadenoma (benign breast tumour). X-ray fluorescence (XRF) has been used by Rizk and Sky-Peck (1984) to study paired samples, while total reflection XRF was utilised by Majewska et al. (1997) to compare benign to malignant breast tumours. More recently, studies by our group have shown statistically significant changes in levels of copper, iron, zinc and potassium in breast tissue, these changes being associated with cancer (Geraki et al., 2002, 2004). The later studies were carried out using synchrotron radiation to excite an XRF response from elements of interest and utilising calibration samples for the quantification of the elemental concentrations.

There are several reasons for investigating elemental concentrations in cancers depending on the roles the elements play. Three trace elements of interest in this work were iron, copper and zinc. Copper and zinc are known to act as catalysts for antioxidant enzymes like superoxide dismutase 1 (SOD 1 and 2). These enzymes have a role to play in the defence against disease. However, copper can also act as a catalyst for the production of hydroxyl radicals that are linked to tissue destruction and has an important role in angiogenesis. Iron is necessary for the growth of cancer and transporters for its uptake are often upregulated in cancer. Zinc is a co-factor for a group of

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enzymes that protects tumours from acidosis (carbonic anhydrases), which are also potential therapy targets. Our previous studies have shown that typical concentrations of these elements in breast tumours are approximately 1, 7 and 15 ppm for Cu, Zn and Fe, respectively. Potassium cannot be studied with the samples used because the fixing process removes this element.

This paper describes the study of the spatial distribution of a number of trace elements within breast cancer with a primary focus on the role of copper, and secondary focus on iron and zinc. Recent evidence has indicated that copper has a key role to play in reducing angiogenesis and tumour growth (Lowndes and Harris, 2004). Copper-binding drugs can have a prolonged stabilisation effect in advanced cancer patients and are being studied in phase I and phase II trials. Although the exact mechanism is not clear, it appears that the anti-growth function of copper chelators is mainly due to the fact that they induce the inhibition of angiogenesis, the creation of blood supply that sustains a growing tumour (Pan et al., 2002). Our group is also interested in the role of copper in the function of SOD. SOD is an enzyme involved in many diverse processes; the emphasis in relation to breast cancer is mainly due to its importance in endothelial signalling and therefore tumour proliferation. Both copper and zinc are essential for the physiological function of SOD, which leads to the utilisation of copper chelators as a means of disrupting the function of the enzyme. Furthermore, caeruloplasmin, a key copper binding protein that was previously thought to be produced only in the liver, is another copper-related pathway of interest. It has been shown that in certain groups of breast cancers, the oestrogen-receptor-negative tumours that show the more aggressive phenotype, express the RNA for this gene (Sotiriou et al., 2003). An investigation of lysyl oxide along with SOD expression has found that both these copper-dependent enzymes are highly expressed in tumour cells compared to normal cells in breast cancer.

Understanding which cell types contain copper at the highest level and differences between individual tumours based on other biology such as oestrogen receptor, will be helpful in future development of copper-chelation therapy. In particular, it would be useful to differentiate between the metal content of the proliferating compartment of the tumour cells, cells near blood vessels and the vessels themselves. Also, inflammatory cells such as macrophages are known to be high in iron content, and it would be of interest to see if they are also a source of copper that could further activate the production of angiogenic factors.

In this paper we show a method of using a synchrotron-based micro-probe to determine the localisation of trace elements in breast tumours at a cellular level. Common techniques of histological staining can be either inapplicable or not sensitive enough for these cases, for example immunohistochemistry could not be used to stain caeruloplasmin because, as it is a plasma protein, it is spread across the tissue section.

2. Methodology

Measurements were made on individual samples approximately 10 μm thick (mounted on 4 μm thick ultralene film) of a formalin-fixed paraffin-embedded tissue microarray of human primary-invasive breast carcinomas. Measurements made on the ultralene film showed no signal from the elements of interest. The samples were obtained from the Cancer Research UK Tumour Pathology Group, University of Oxford, Nuffield, Department of Clinical Laboratory Sciences, John Radcliffe Hospital, Oxford. Each section on the array was 1.0 mm diameter and 10 μm thick.

The data were collected using the synchrotron X-ray fluorescence microprobe (SY-XRF) at Hasylab, Beamline L (HYMO) which is a powerful tool for simultaneous multitrace element analysis of microsamples. The white beam of a bending magnet source is monochromatised by a double-multilayer-monochromator with a bandpass $\Delta E/E \sim 2\%$. The beam is focused by a polycapillary half lens (X-ray Optical Systems, Inc.) which provides a beam of 10–25 μm diameter, depending on energy. For the present study, the excitation energy was set to 12 keV, which is a compromise of maximum Cu signal due to high absorption cross section and minimum size of beam (18 μm FWHM at 12 keV). By using this energy, data are collected for Cl, Ca, K, P, S, Ti, Fe, Cu and Zn. Recent improvements in spectrometer sensitivity and detector count rate capability have improved the limits of detection for XRF. By reducing the measurement time per point, larger areas of sample can be scanned without loss of spatial resolution, which is important, as physiological relevant areas are often several square millimetres. At 20 μm spatial resolution this results in many thousands of points per scan, and in order to keep such scans to an acceptable time (several hours), a continuous scanning mode for collecting data has been developed. In this mode the sample is moved continuously across the beam, not stepwise as in conventional mode. The multichannel analyser opens for a predefined time interval and subsequently the data are read out and written to memory. In addition the signals needed for normalisation, e.g. ionisation chamber signals, detector deadtime, ring current, are saved. The sampling time for each data point is 5 s, which will allow samples to be scanned at the rate of approximately 6 h each. The samples are supported on an XYZ table with a reproducible positioning of about 0.5 μm. The fluorescence signal is recorded using a Peltier cooled energy dispersive Si drift detector (Radiant VORTEX). The data are analysed using AXIL, a programme that fits the element Kα and Kβ peaks under consideration, taking account of line overlaps and subtracts the background. Automatic 2D scans are programmed and performed and trace element distribution maps are obtained with a matrix size of 63×58 pixels resulting in an area of 1.26×1.16 mm.