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Cancerous tissues analyzed by SRIXE

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Abstract

Synchrotron radiation induced X-ray emission (SRIXE) analysis of trace element (TE) concentrations in prostate tissues shows the significant differences between elemental concentrations in cancer and healthy parts of tissues. The analysis enables obtaining additional information about the prostate cancer, almost the most dangerous disease of the recent days.

The cancer tissue sections were obtained after radical removal of the organ from the patient's body. The samples were histologically examined and classified as well as the Gleason score was determined. After histological examination, the samples were irradiated with monochromatic X-ray beam at the beam line L at HASYLAB in DESY.

Our investigation proved that the elements such as Mn, Fe, Co, Ni which are involved in cancerogenesis appeared on elevated concentration levels in cancerous part of the tissues in comparison to non-cancerous parts. This study may also indicate that the advancement of prostate cancer is connected with the content of copper, zinc and iron.

Keywords: Trace elements; Prostate; X-ray fluorescence; X-ray spectroscopies; Synchrotron radiation

1. Introduction

Cancer as disease and cancerogenesis as biological process has been the subject of enormous number of studies: clinical and experimental, based on different philosophy and technical approaches. Although at present genetics on one side and immunology on the other (both assisted by biochemistry) seem to be leading, the physical approach (the methods of nuclear physics and X-ray spectroscopy) offers also some valuable possibilities. Also, it is known that elemental composition of cancerous versus normal tissues was subjected to numerous analyses including physical methods but we decided to follow the track again for three reasons:

First: Some of the methods, based on synchrotron radiation, X-ray fluorescence and ion beams irradiation allow obtaining the high sensitivity detection of trace elements in the tissues combined with good correlation to organ structure.
Second: The frequency of tumor selected for investigations—carcinoma of the prostate is the most common cancer in males and is the second leading cause of death in the world.
Third: Some time ago, it was believed that benign prostatic hypertrophy (nodular hyperplasia) may be regarded as change leading to the higher cancer risk. This has not been proved yet but the raised level of androgens (di-hydroxy-testosterone) formed locally from testosterone by 5 alpha reductase-2 is believed as the main cause of nodular hypertrophy. Androgens elevation is regarded also as a main pathogen of prostate cancer.

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Differences in elemental composition of normal and transformed (cancer) tissues were discussed in a number of papers [1–6]. Others tried to find the changes coexisting with carcinogenic process. However, the most of the studies concern the levels of elements in plasma (blood) [7,8]. Much less observations have been made on tissues especially using physical techniques that permit good localization of trace elements (TE) in organs.

We decided to concentrate our study mainly on the three elements: Fe, Cu and Zn. This approach comes from the following reasons:

(i) it was found that relation between the levels of Fe, Cu and Zn can be explained on the basis of biochemistry and physiology [3,7,9];
(ii) the elements from the list are mostly bound to proteins what minimize the dislocation during applied procedures: sample preparation and the measurements;
(iii) in prostate tissue one may observe different levels of these elements depending on different stages of tumor progress according to Gleason classification. This offers broader field for drawing conclusions [10].

2. Material

The samples were obtained from six patients operated due to prostate cancer disease. Two patients were classified as hyperplasia (HP) and four as CA (adenocarcinoma). The patient's age was ranged from 53 to 63. After operations, samples were frozen in liquid nitrogen, and then cut into 14 μm-thick sections with a cryo-microtome. Those sections were placed on a 2.5 μm Mylar foil. Adjacent sections were put on a microscopic glass for later histological examination. Those sections were placed on poly-L-lysine coated slides, air dried, fixed in 4% phosphate buffered paraformaldehyde and routinely stained with Meyer's hematoxylin and 0.5% aqueous eosin. Olympus BX-50 microscope was used to choose the right area for TE analysis. The samples were classified into groups: non-cancerous and cancerous based on patomorphological examination. The Gleason score was also determined for each cancerous sample. From each sample several tissue sections were prepared—in total 37 sections were prepared: 8 adenocarcinoma with Gleason score 3 (CA3), 8 adenocarcinoma with Gleason score 5 (CA5) and 21 hyperplasia (HP).

Examples of prostate stained tissue sections are presented in Figs. 1–3. The marked frames in Figs. 1 and 2 show the areas from which the selected points were analyzed.

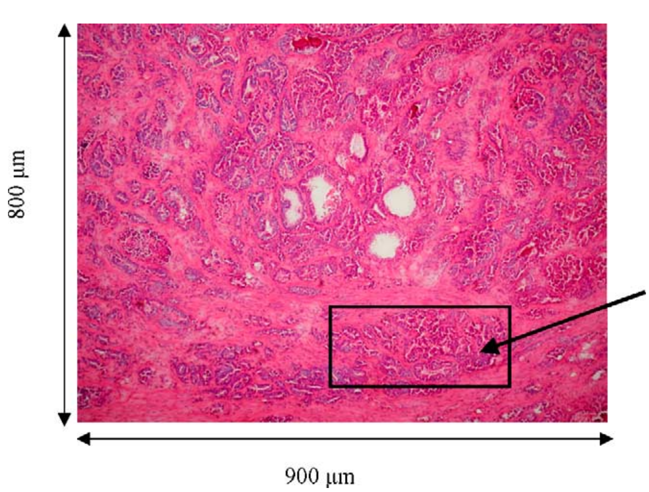

Fig. 1. Histological view of a prostate adenocarcinoma with Gleason score 5 tissue with marked area of the TE measurements. The arrow points to the cancerous cells.

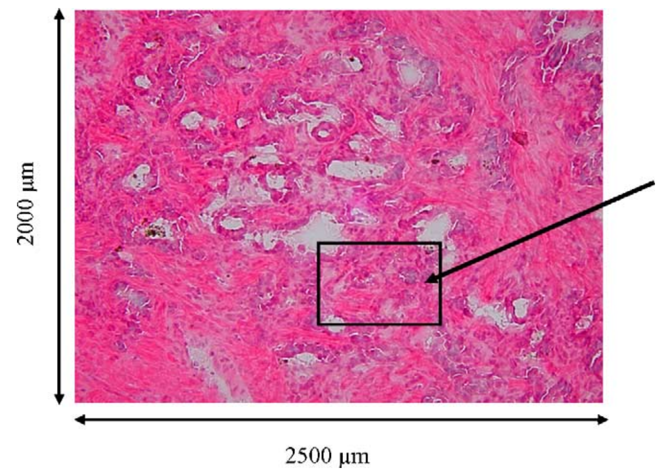

Fig. 2. Histological view of a prostate adenocarcinoma with Gleason score 3 tissue with marked area of the TE measurements. The arrow points to the cancerous cells.

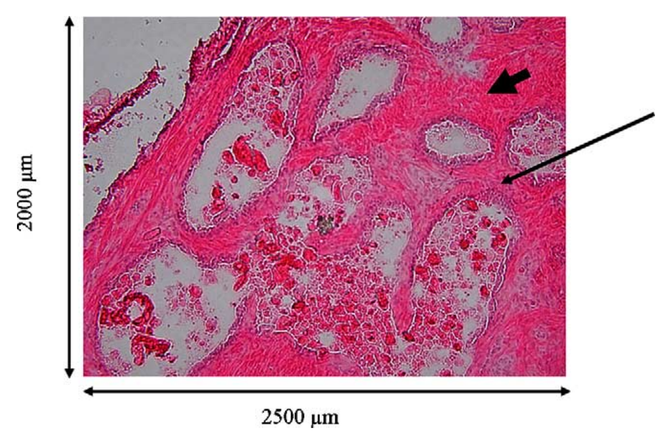

Fig. 3. Histological view of a prostate hyperplasia tissue. The arrow points to the overgrown gland and arrowhead points to the stroma.

3. Experimental

Synchrotron radiation induced X-ray emission (SRIXE) is now a well-known experimental technique, which theory and details have been widely discussed in several papers [12,13].

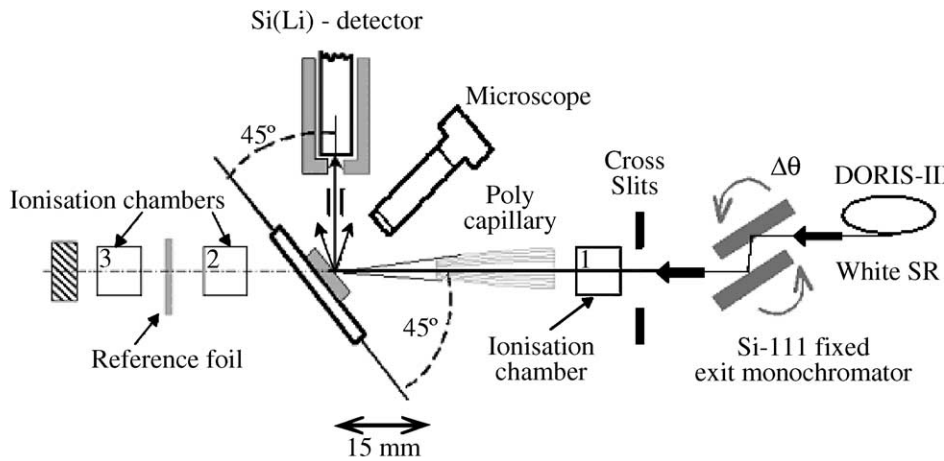

Fig. 4. Schematic diagram of the L-beam line setup [14].

Since synchrotron delivers beam of high flux and brightness no pre-concentration procedure for sample preparation is required for trace element analysis. The use of synchrotron radiation beam enables the analysis of low concentrated elements that exist in biological materials. An important aspect of the SRIXE technique is the ability to make TE measurements with excellent spatial resolution. Using capillary system the beam could be focused down even to 0.1 μm, while with slits set-up the collimated beam size was about 10–20 μm in *X* and *Y* directions.

In this case, the measurements were carried out on the L-beam line at the HASYLAB, DESY (Germany). The spectra were recorded using a conventional Si(Li) detector with the energy resolution of 140 eV for 5.9 keV. The beam size was set down to 100 μm × 100 μm in order to obtain the average concentration level of different tissue structures, and then the capillary was used to obtain spectra from single points. The beam size of 20 μm in diameter was achieved. The SRIXE measurements as point analyses were performed with the monochromatic beam of 17 keV. The spectra were usually collected for 300 s live time for single point analysis.

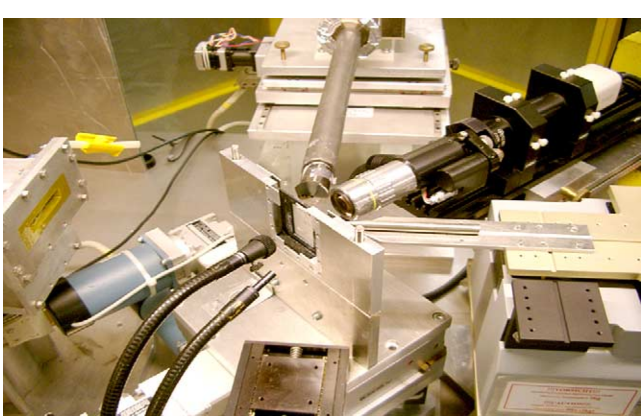

Fig. 5. Photo of the experimental table viewing sample holder arrangement at the L-beam line in HASYLAB.

Fig. 4 shows a schematic diagram of L-beam line set-up at HASYLAB while Fig. 5 presents a photograph of the experimental table viewing the sample holder.

For TE determination two standards were prepared [5], standard MULT I composed of the mixture of nitric metals such as: Ca, V, Fe, Zn, As, Pb, Sr, Zr, Mo and Cd and standard MULT II included: K, V, Mn, Fe, Zn, Hg, Pb, Sr, Zr and Mo. Metal concentrations in those standards were at the level of 10 μg/g.

4. Results

The SRIXE spectra were analyzed with simple and fully controllable program AXIL. Table 1 presents the calculated mean values of TE concentrations. One should emphasize that the mean values were calculated from point analyses taken always on the same tissue structure for selected samples with the beam size of 100 μm × 100 μm.

Table 1
Mean values and standard deviations of trace element concentrations determined in cancerous and non-cancerous prostate tissues sections

Element	Cancerous tissues		Non-cancerous tissues
	CA5 (Gleason 5)	CA3 (Gleason 3)	HP
Cl	1830 ± 190	1700 ± 60	1800 ± 190
K	3620 ± 80	2560 ± 470	1475 ± 250
Ca	410 ± 43	150 ± 13	120 ± 24
V	2.02 ± 0.21	0.11 ± 0.01	0.41 ± 0.06
Cr	0.87 ± 0.07	0.33 ± 0.06	1.30 ± 0.10
Mn	3.45 ± 0.52	2.74 ± 0.27	1.8 ± 0.2
Fe	**114.50 ± 19.20**	**24.1 ± 3.5**	**52 ± 4**
Co	5.4 ± 0.6	4.7 ± 0.4	3.8 ± 0.3
Ni	5.1 ± 0.8	6.3 ± 1.2	4.5 ± 0.4
Cu	**12.1 ± 1.5**	**15.3 ± 2.2**	**12.7 ± 2.5**
Zn	**174 ± 16**	**176 ± 19**	**158 ± 28**

The concentration values and standard deviations are given in (μg/g). CA5 stands for tissues classified as adenocarcinoma with Gleason score 5, CA3 stands for tissues classified as adenocarcinoma with Gleason score 3 and HP stands for tissues classified as hyperplasia.

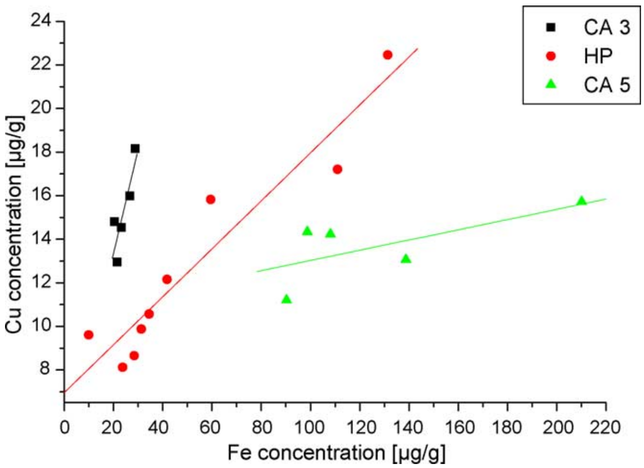

Fig. 6. Correlation between Cu and Fe concentration in (µg/g) for different type of samples.

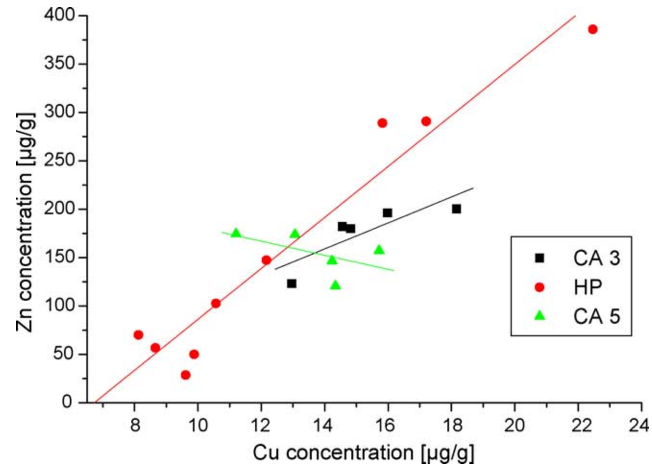

Fig. 8. Correlation between Zn and Cu concentration in (µg/g) for different type of samples.

The analysis of elemental concentrations shows that potassium concentration was the lowest in hyperplastic prostate, the highest in the case of adenocarcinoma with Gleason score 5 and intermediate in adenocarcinoma with Gleason score 3. The results obtained enable also the calculation of correlation between the element concentrations. In this case, the beam size was set down to 20 µm in diameter by the capillary. The most interesting correlation has been found between copper and iron. In all samples one can easily observe positive correlation between those elements in all types of tissue but trends are different. There is very narrow range of iron concentration for samples marked as CA3 but for samples marked as CA5 seems to be broad while corresponding copper concentration range seems to be almost the same (there is a slight shift towards higher copper concentrations for samples CA3). The Cu–Fe correlation for samples marked as HP is about 1 and the concentrations covers broad ranges which include concentration ranges for CA3 and CA5. Fig. 6 shows the results obtained along with the fitted trends for those data.

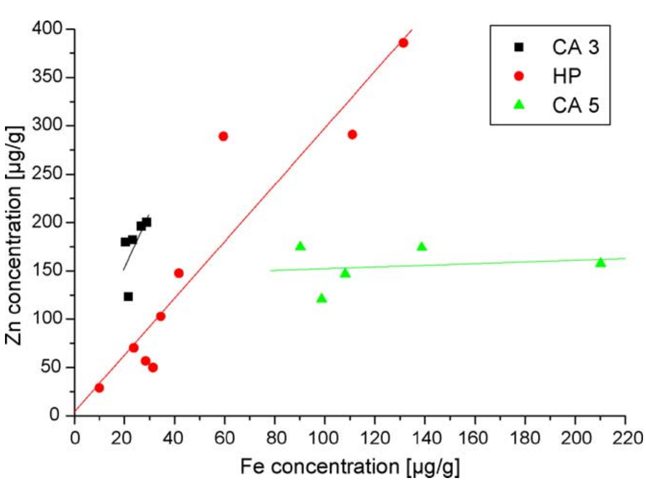

Fig. 7. Correlation between Zn and Fe concentration in (µg/g) for different type of samples.

The results obtained for trace element concentrations determined in cancerous tissues with Gleason score 3, in cancerous tissues with Gleason score 5 and in non-cancerous (benign hyperplasia) show the positive correlation between Cu and Fe and between Zn and Fe as shown in Figs. 6 and 7, respectively. In the case of relations between Zn and Cu, there are positive correlations for tissues classified as benign hyperplasia and cancerous with Gleason score 3 (CA3). The negative correlation between Zn and Cu has been observed in the case of cancerous tissues with Gleason score 5 (CA5) as shown in Fig. 8.

5. Discussion

The differences in TE concentrations, partially, may result from different tissue composition of investigated prostate. Good example is potassium level, lowest in hypertrophy (where both stroma and epithelial tissues are effected) at highest at adenocarcinoma with Gleason 5 where proliferating tumor cells dominate. As it is well known potassium concentration is much higher in cells than in extra-cellular substance. Significant concentration differences between cancerous and non-cancerous tissues are also evident for the other elements. The elements such as Mn, Co, Ni are involved in cancerogenesis and are usually appeared on elevated levels. In our study, they were also found at higher levels in cancerous part (CA5, CA3) of the tissues in comparison to non-cancerous part. The importance of this will be a subject of further analysis.

Concentration of iron increased with the Gleason scores. This may be explained by enlarged amount of blood what results from known process of tumor angiogenesis.

The differences in the line slopes (in Fig. 6) may result from different erythrocytes content (Fe)/plasma ceruloplasmin (Cu) ratios caused by exovasation of blood and plasma formation of crystals containing Fe atoms.

In Figs. 6–8, it is visible that ratios of Cu/Fe, Zn/Fe and Zn/Cu differ depending on investigated samples. In prostrate hypertrophy, the Zn/Cu ratio seems to be constant (increase of Zn concentration parallels increase of Cu concentration), in adenocarcinoma with Gleason 5 concentration of these elements correlates negatively, in adenocarcinoma with Gleason 3 slope of ratio trend is between values for CA5 and HP. It has been reported [8,9,11] that differences of these two elements concentrations in tissue and in blood plasma are observed. Our investigation may also indicate that the advancement of prostate cancer is associated with the content of these two elements.

Information about concentration levels of trace elements in cancerous and non-cancerous tissue [15] may verify the hypothesis about influence of trace elements on cancer disease in human population.

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References

[1] B. Florianczyk, J. Osuchowski, R. Kaczmarczyk, T. Trojanowski, Folia Neuropathol. 41 (1) (2003) 11–14.
[2] H. Tashiro, T. Kawamoto, T. Okuba, O. Koide, Biol. Trace Elem. Res. 95 (1) (2003) 49–63.
[3] S. Chan, B. Gerson, S. Subramanium, Clin. Lab. Med. 18 (4) (2004) 673–685.
[4] W.M. Kwiatek, B. Kubica, C. Paluszkiewicz, M. Galka, J. Alloys Compd. 328 (2001) 283–288.
[5] W.M. Kwiatek, A.L. Hanson, C. Paluszkiewicz, M. Galka, M. Gajda, T. Cichocki, J. Alloys Compd. 362 (1–2) (2004) 83–87.
[6] K. Geraki, M.J. Farquharson, D.A. Bradley, Phys Med. Biol. 49 (1) (2004) 99–110.
[7] T. Wu, C.T. Sempus, J.L. Freundenheim, P. Muti, E. Smit, Ann. Epidemiol. 14 (3) (2004) 195–201.
[8] M.I. Yilmaz, K. Saglam, A.B. Sonmez, Biol. Trace Elem. Res. 98 (1) (2004) 13–19.
[9] M. Kucharzewski, J. Braziewicz, U. Majewska, S. Gozdz, Biol. Trace Elem. Res. 93 (1–3) (2003) 9–18.
[10] K. Jafari-Khouzani, H. Soltanian-Zadeh, Trans. Biomed. Eng. 50 (6) (2003) 697–704.
[11] S.B. Nayak, V.R. Bhat, D. Upadhyay, S.L. Udupa, Indian J. Physiol. Pharmacol. 47 (2003) 108–110.
[12] K.W. Jones, in: R.E. Van Griken, A. Markowicz (Eds.), Handbook of X-ray Spectrometry, Marcel Dekker, New York, 1992, p. 411.
[13] W.M. Kwiatek, Acta Phys. Pol. A 86 (1994) 695–703.
[14] http://www-hasylab.desy.de/science/annual_reports/2001_report/part1/intern/5720pdf.
[15] W.M. Kwiatek, T. Drewniak, M. Gajda, M. Gałka, A.L. Hanson, T. Cichocki, J. Trace Elem. Med. Biol. 16 (2002) 155–160.