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An evaluation study of trace element content in colorectal liver metastases and surrounding normal livers by X-ray fluorescence

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Abstract *Background* Trace elements are involved in many key pathways involving cell cycle control. The levels of trace metals such as iron, copper, and zinc in colorectal liver metastases have not previously been assessed. *Methods* The trace element content in snap-frozen cancerous liver tissue from patients who underwent liver resection for colorectal liver metastases was compared with the normal surrounding liver (distant from the cancer) using X-ray fluorescence (XRF). *Results* X-ray fluorescence was performed on a total of 60 samples from 30 patients. Of these 29 matched pairs (of cancer and normal liver distant from cancer from the same patient) were eligible for univariate analysis. Iron (0.00598 vs. 0.02306), copper (0.00541 vs. 0.00786) and zinc (0.01790 vs. 0.04873) were statistically significantly lower in the cancer tissue than the normal liver. Iron, copper, and zinc were lower in the cancer tissue than in the normal liver in 24/29 (82.8%), 23/29 (79.3%), and 28/29 (96.6%) of cases respectively. Multivariate analysis of the 60 samples revealed that zinc was the only trace element decreased in the cancer tissue after adjusting for the other elements. Zinc levels were not affected by any of the histopathological variables. *Conclusion* Iron, copper, and zinc are lower in colorectal liver metastases than normal liver. An investigation into the pathways underlying these differences may provide a new understanding of cancer development and possible novel therapeutic targets.

Keywords Trace elements · Iron · Zinc · Copper · Metastasis · Liver · X-ray fluorescence spectrometry

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Background

Trace elements are involved in many key pathways involving cell cycle control. Mobilisable iron increases the production of oxygen free radicals (Barbouti et al. 2001) and promotes tumorigenesis (Rezazadeh and Athar 1997; Rezazadeh et al. 2005). Mobilisable copper also causes oxidative damage (Ferretti et al. 2003). Zinc induces apoptosis (programmed cell death) in prostate cells (Costello and Franklin 2006). However, other studies have shown that zinc deficiency can also induce apoptosis (Chai et al. 1999). High levels of zinc are required for the nuclear functions during early differentiation

(Beyersmann and Haase 2001). Zinc is a co-factor or an essential component of nearly 300 enzymes including metallothioneins, superoxide mutases and plays a role in anti-oxidation, immune function and inflammation (Tapiero and Tew 2003).

Previous studies in patients with liver metastases suggest that there may be differences in trace elements between metastatic liver cancers and normal tissues (Olson et al. 1958; Wright and Dormandy 1972; Gurusamy and Davidson 2007) with all three elements lower in cancer tissue than in surrounding normal livers. However, in the previous studies, only the mean values between cancers and surrounding normal livers were compared and the difference in levels between these tissues in the individual patient was not considered. Also, previous studies measured the trace elements in liver metastases from different primary cancers. The metal content was different in different types of primary tumors metastasizing to the liver (Roguljic et al. 1980). Trace element concentration of colorectal liver metastases and normal surrounding liver has not been assessed previously. The aim of this study is to compare the trace element content in colorectal liver metastases with that of normal surrounding liver from the same patient.

Methods

The samples were obtained from patients who underwent liver resection. Ethical approval was obtained and consent for use of tissue for research was obtained from all patients. Two samples, one from the cancer and another from the normal appearing liver (from the resected tissue) distant from the cancer, were obtained from each patient and were snap-frozen in liquid nitrogen and stored at −80°C. The samples were mounted on sample holders free from iron, copper and zinc. Extreme care was taken to avoid contamination of these samples with any of the metals.

The measurement of X-ray fluorescence (XRF) is a method of determining the elemental content in a tissue by using the property of the emission of X-rays of specific, characteristic energies by each element when the elements are exposed to an X-ray source. XRF has been used for measuring the trace elements in different human tissues including liver, with a high level of accuracy of between 98 and 99% (Carvalho and Marques 2001) and repeatability of between 98 and 99% (Milman et al. 2000; Laursen et al. 2001). The measurements were carried out at the European Synchrotron Radiation Facility (ESRF 2007) at Grenoble, France on beam line BM28. The energy of the synchrotron beam was tuned to 13 keV which enables the XRF response from Fe, Cu and Zn to be recorded. The measurement time for each sample was approximately 20 min. The results were recorded in the form of an energy spectrum which included the response peaks from the metals plus the scattered incident radiation peak. The resulting spectrum was analyzed using SPSS Peakfit 4.0 and the total counts in each peak (area under the peak) calculated for each element and the scatter peak. The area of the scatter peak was used to normalize the XRF response peaks. The mean spectrum for all the samples is shown in Fig. 1.

Histological analysis of the same tissue samples was performed using haemotoxylin and eosin stain. Visual assessment of the percentage of necrosis and percentage of tumor in each section was noted. Statistical analysis was performed using SPSS 14.0. Univariate analysis using Wilcoxon-matched pair test was performed. A P value of <0.05 was considered statistically significant. The coefficient of variance was calculated to compare the variation in the different trace metals. The correlation between the trace element content between the cancer tissue and the normal liver was calculated using the Spearman's correlation coefficient. Multivariate analysis using logistic stepwise (forward and backward) regression

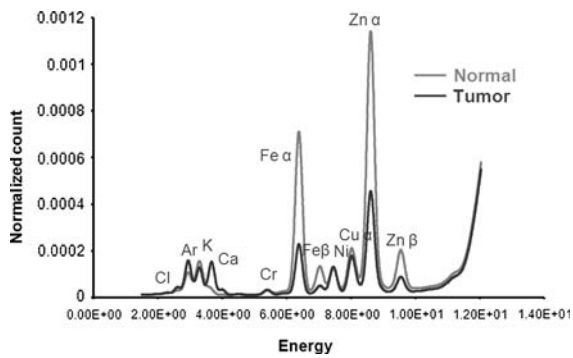

Fig. 1 Average X-ray Fluorescence Spectrum for normal and cancer samples. Alpha and beta energies of the same element correspond to the energy released when the electron jumps from the L to K shell and from the M to K shell respectively

analysis was also performed to determine if the individual metal content varied after adjusting for the other metals. Further multivariate linear regression analysis was performed to determine if the metal content varied with histological variables such as subtypes or grade.

Results

The XRF measurements were performed on a total of 60 samples from 30 patients. One of the samples labeled as normal was found to be adenocarcinoma on histopathological examination. Of the 29 matched pairs, one was poorly differentiated, six were necrotic tumors, two were mucinous adenocarcinoma and the remaining 20 were well or moderately differentiated adenocarcinoma. The iron, copper and zinc followed a non-parametric distribution.

Univariate analysis

All three metals (iron, copper and zinc) were statistically significantly lower in the cancer tissue than in the normal surrounding liver. Zinc was lower in the cancer tissue compared to the normal surrounding liver in 28/29 (96.6%) of cases. Iron and copper were lower in the cancer tissue than in the normal liver in 24/29 (82.8%) and 23/29 (79.3%) of cases respectively. The median area under the curve (AUC) with the range for each group is given in Table 1. In cancer tissues, the AUC ranged between 0.000146 and 0.03932 for iron; between 0.00442 and 0.02681 for copper; and between 0.01137 and 0.03463 for zinc. In normal livers, the AUC ranged between 0.00681 and 0.31102 for iron; between 0.00579 and 0.01473 for copper; and between 0.02848 and 0.08986 for zinc. All figures represent normalized peak counts for each element. The box plots of the median AUC of these trace elements in cancer and normal liver is shown in Figs. 2, 3, 4. The coefficient of variance showed that normal liver copper and zinc; and cancer zinc were less variable across individuals than cancer copper, normal liver iron, and cancer iron. There was no statistically significant correlation between the trace metal content in cancer and in normal livers from the same patient (Zinc levels in normal and cancer tissues are shown in Fig. 5).

Multivariate analysis

For multivariate analysis, all the 60 samples were included (31 cancer samples and 29 normal liver samples). In the two cancer samples from the same patient, the zinc levels were very similar (0.021344 and 0.022616), while that of iron and copper varied by a factor of 4.6 times and 1.5 times. By using multiple regression analysis of all three trace elements, it was found that zinc was the only trace element included in the model indicating significant difference in the zinc levels between the cancer tissue and normal liver after adjusting for other elements. The variation between the cancer and the normal livers was so high that by substituting the zinc AUC in the equation obtained from logistic regression, it was possible to predict whether the tissue was cancer or normal liver with a sensitivity of 100% and a specificity of 90%. Further linear regression analysis showed that the zinc levels were not affected by any of the histopathological variables including the subtype, grade, the percentage of the slide occupied by the tumor (tumour slides contained some connective tissue and/or adjacent normal liver tissue) and the percentage of the slide occupied by necrosis.

Discussion

This study has confirmed significant differences in the tissue levels of trace elements between colorectal liver metastases and normal tissue. All three metals but particularly zinc, were significantly lower in the cancer tissue than the normal liver (distant from cancer).

There is wide variation in the levels of the trace elements reported in the livers from normal individuals (Gurusamy and Davidson 2007) reported in different studies. Some reasons for the variations include the composition of the population studied and the method used for measurement of the trace elements (Gurusamy and Davidson 2007).

Trace elements have previously been measured in liver metastases and compared with levels in the normal liver (Olson et al. 1958; Wright and Dormandy 1972). In this study, we have attempted to

Table 1 Metal AUC in the tumor and surrounding tissue[a]

Metal	Cancer				Normal liver			
	Median	Standard deviation	Range	Coefficient of variance	Median	Standard deviation	Range	Coefficient of variance
Iron	0.00598	0.00763	0.00146–0.03932	0.90329	0.02306	0.05605	0.00681–0.31102	1.41245
Copper	0.00541	0.00463	0.00442–0.02681	0.64103	0.00786	0.00218	0.00579–0.01473	0.26101
Zinc	0.01790	0.00583	0.01137–0.03463	0.30094	0.04873	0.01325	0.02848–0.08986	0.25697

[a] All three statistically significant by Wilcoxon-matched pair test. All figures are normalized peak counts

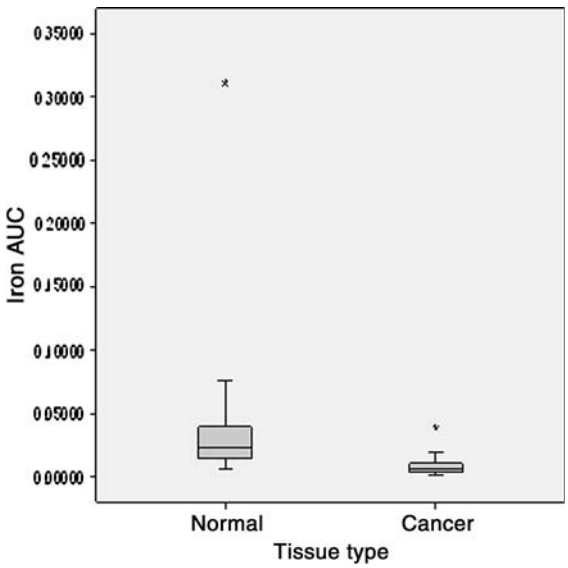

Fig. 2 Iron content in tumour tissue and normal surrounding liver. AUC = Area under the curve of iron content in tumour tissue and normal surrounding liver

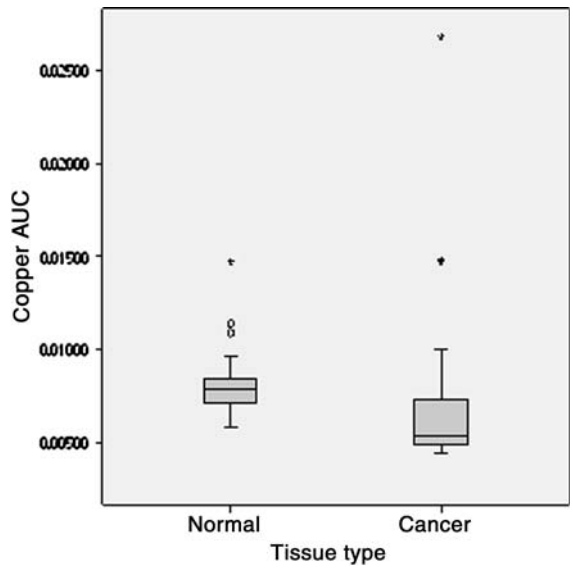

Fig. 3 Copper content in tumour tissue and normal surrounding liver. AUC = Area under the curve of copper content in tumour tissue and normal surrounding liver

overcome the defects in the previous studies by comparing the levels of trace elements in both the cancer and normal liver tissue of individual patients. We found that zinc is lower in tumors than normal liver in 97% of cases. The iron and copper levels were lower in tumors than normal liver in around 80% of cases. The copper–zinc ratio was higher in the tumors than normal livers in 97% of cases but this was chiefly due to lowered zinc. Imbalance in plasma copper/zinc ratio has been associated with many other diseases including infectious diseases (Van Weyenbergh et al. 2004), hypertension (Canatan et al. 2004) and other cancers (Cunzhi et al. 2003).

X-ray fluorescence is an established method of measuring trace elements with very good repeatability (Milman et al. 2000; Laursen et al. 2001). The variation between the cancer and the normal livers is so high that by substituting the zinc AUC in the equation obtained from logistic regression of 50 samples (not shown in the results), it was possible to predict whether the tissue was cancer or normal liver in all the remaining 10 samples. This indicates that the variability in zinc content between cancer and normal liver is much greater than the variability in the trace element content of cancers (and normal livers) of individual patients. The low value of coefficient of variance for zinc in both cancer and normal liver tissues also suggests a low variability in tissues of the same type.

It was not possible to calculate the absolute concentration. Hence, it is not possible to compare the trace metal concentration measured by us with those in the literature. As correlation of trace elements with histological variables was one of the

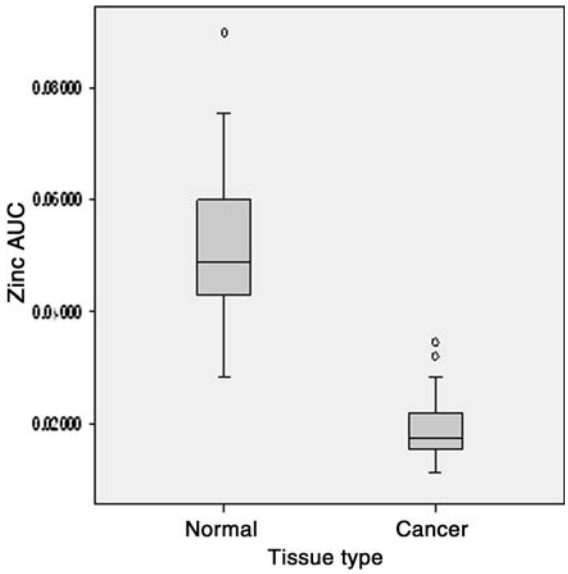

Fig. 4 Zinc content in tumour tissue and normal surrounding liver. AUC = Area under the curve of zinc content in tumour tissue and normal surrounding liver

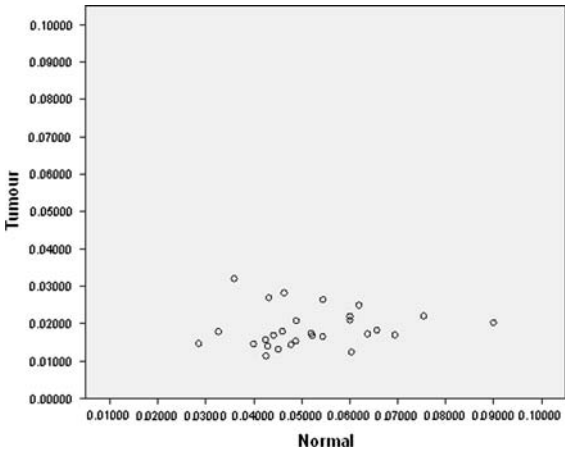

Fig. 5 Zinc content of colorectal liver metastases and normal surrounding liver (normalized counts). Correlation between the zinc content of colorectal liver metastases and normal surrounding liver in each individual

objectives of the research, methods which consume the tissues such as atomic absorption spectroscopy were not used. However, atomic absorption spectroscopy can be used to further validate XRF. It was also not possible to repeat the measurements on the same sample, measure intra-tumoral variation and inter-tumoral variation in individual patients because of the time required for analysis. Given the clear data demonstrating the differences, further studies will analyze intra-tumoral and inter-tumoral variation and measure the absolute concentration of the trace elements.

There was no statistically significant correlation between the trace metal content of cancer tissue and normal livers from the same patient. This suggests that the lowered trace metal content in cancers is unlikely to be due to environmental, dietary factors or due to alterations in the level of absorption of these metals from the gastrointestinal tract.

Intracellular zinc levels are maintained through a balance of two families of proteins ZIP and ZnT. The ZIP family is a group of 14 zinc transporters which increase the intracellular zinc (Eide 2006) by transporting the zinc into the cell cytoplasm from outside the cell or from inside the cell organelles. ZnT family is a group of nine zinc transporters which decrease the intracellular zinc (Eide 2006) by transporting the zinc from the cell cytoplasm to outside the cell or into cell organelles. The different members of each family are situated at different locations (Eide 2006). ZIP1 protein (a member of ZIP family) and its gene expression have been reported to be decreased in prostate cancer tissue compared to normal prostate (Franklin et al. 2005). There are no previous reports on zinc transporters in primary or secondary liver cancers but variation in their levels in colorectal liver metastases may explain the findings in this study. Increased utilization of zinc by the fast growing tumour tissues is another possible reason for the observation.

Similar to zinc, iron content is lower in the liver metastases compared to the surrounding non-tumor liver tissues. Iron content has also been found to be decreased in other cancers including primary liver cancers (Tashiro et al. 2003). Higher expression of transferrin receptors (iron influx transporter) has been shown in colon cancer cells growing in the murine liver (Shinohara et al. 2000) but this has not been investigated in colorectal liver metastases. Iron exporter FPN (Ferroportin-1 or Iron-regulated transporter 1) is increased in colorectal cancers (Brookes et al. 2006). Possible explanations for low iron content in liver secondaries could be the increased iron exporters in secondary cancers (greater than the increase in the transferrin receptors) and/ or depletion of iron by the fast growing tumour tissues.

Copper content is also lower in liver metastases compared to the surrounding normal tissues. Studies

have shown that the copper efflux-transporters are increased in certain poorly differentiated cancers (Nakayama et al. 2002; Aida et al. 2005). An increase in copper efflux transporters could explain the decreased copper content of secondary liver tumors. Increased utilization of copper by the fast growing tumour tissues is another possible reason for the observation.

There is a paucity of research analyzing the reason for these differences between cancer and normal livers and the role of the trace elements in the metabolic pathways of colorectal liver metastases. An investigation into the pathways underlying these differences may provide a new understanding of cancer development and possible novel therapeutic targets.

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