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CLINICAL STUDIES

Analysis of iron, zinc, selenium and cadmium in paraffin-embedded prostate tissue specimens using inductively coupled plasma mass-spectrometry

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Abstract

Formalin-fixed paraffin-embedded (FFPE) tissue specimens represent a valuable and abundant resource of pathologic material for various biomedical studies. In the present study, we report the application of high-resolution inductively coupled mass-spectrometry (ICP-MS) for quantification of Fe, Zn, Se and Cd in FFPE prostate tissue. These elements have a possible role in the development of prostate diseases: while Zn and Se are needed for a healthy prostate, Cd shows multiple toxic and carcinogenic effects. Excessive accumulation of Fe induces the production of highly reactive hydroxyl radical species, which may play a role in cancer etiopathogenesis. To assess whether the levels of these metals in the FFPE prostate tissue represent their original content, we compared their levels with those in the fresh tissue (on dry weight basis) in samples obtained from 15 patients. We found that in FFPE tissue, the recoveries of Se, Fe, Cd and Zn were progressively decreased, $97 \pm 11\%$ ($r = 0.88$), $82 \pm 22\%$ ($r = 0.86$), $59 \pm 23\%$ ($r = 0.69$) and $24 \pm 11\%$ ($r = 0.38$), respectively. Thus, the use of correction factors, determined as $k = 0.16$ for Se, $k = 0.20$ for Fe, $k = 0.27$ for Cd and $k = 0.67$ for Zn, is required to estimate the retrospective levels of these elements in the parental non-processed fresh (wet) prostate tissue. The technique used in this study enables the analysis of archival FFPE prostate tissue for the concentrations of Fe, Zn, Se and Cd to study association between the levels of these metals and prostate disease.

Keywords: Prostate; Paraffin-embedded tissue; ICP-MS; Trace metals; Cadmium

Introduction

Formalin-fixed paraffin-embedded (FFPE) tissue, in well-characterized archival material, represents a valuable and abundant resource of material for various biomedical studies. Though this tissue is modified by the fixation and embedding process (leading to partial degradation, potential leakage from the tissue and change in structure of certain components), it remains one of the most important (and sometimes the only) source material available for chemical and analytical

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toxicological studies. A large number of methods, such as conventional histochemical and immune staining, and PCR (RT-PCR), have been developed to analyze this tissue for proteins, polysaccharides and nucleic acids. The detection of metals involved in various physiological and pathological processes represents another clear objective.

Prostate cancer is the most frequently diagnosed form of noncutaneous cancer in men and the second leading cause of their cancer death in many countries [1]. Three trace elements, Zn, Se and Cd, have been highlighted in the literature in relation to the development of prostate disease [2–4]. Zn is the second most abundant metal in the human body, serving as a cofactor for more than 300 enzymes having various physiological functions [5]. In the prostate, zinc is accumulated at up to 10-fold higher levels (~150 μg/g) than in other organs correlating with the healthy state of the gland, but it has been reported to be several-fold decreased in cancer tissue [6–8]. Cd has distinct antagonist effects with Zn [9,10] and is considered to be a risk factor in the development of prostate cancer in some (but not all) studies [3,11,12]. In addition, cadmium is a well-recognized human carcinogen as established by the International Agent for Research on Cancer [13]. Se incorporates into several types of proteins, serving as antioxidants and is involved in DNA repair [14,15]. A number of studies have suggested the beneficial role of selenium in reducing the risk of cancer; however, at the same time, its excess in the diet (above 0.4 mg/day) can have toxic effects [16,17]. In addition to these elements, iron has been also implicated in prostate disease. Fe is an essential metal in the human body, but at high concentrations it can participate in many biochemical reactions including the producing of highly reactive hydroxyl radicals which are thought to induce cancer in humans [18]. For example, it has been reported that hepatocellular carcinoma develops more frequently with increased iron deposition, whereas a low-iron diet led to smaller tumors in mice [19,20].

The body uptake of the above elements is mainly related to consumption of food, water and anthropogenic occupational exposure. The consumption of Fe, Zn and Se occurs via food (meat, eggs, dairy products and vegetables). As a non-essential element, Cd generally enters the body via smoking, occupational activity, industrial pollution of the environment and contaminated seafood [21–23].

The present work was aimed at developing methodology for quantification of the above elements in FFPE prostate tissue in order to enable exploration of links between these elements and prostate cancer development. One of most important questions to be answered was to determine how these metals in the tissue withstand the embedding/deparaffinization process. The second aim of this study was the selection of an appropriate method for the analysis. Several previously used techniques for analysis of FFPE tissue were based on colorimetric or atomic absorption spectrophotometric determination of a single element, predominantly Fe [19,24–28]. Inductively coupled plasma with mass-spectrometry (ICP-MS) capability has increasingly been used for the multi-elemental determination in trace elements in human biological tissues and fluids, providing high sensitivity and a wide dynamic concentration range. Thus, we have used sector-field-based (high resolution) ICP-MS for determination of Zn, Se, Cd and Fe in fresh non-processed and FFPE prostate tissue specimens.

Materials and methods

Ultra pure water (distilled deionized, 18 MΩ cm) was obtained using Milli-Q system (Millipore Corporation, Billerica, MA, USA) and used for all preparations. Nitric and acetic acids (of "Optima" grade) were purchased from Fisher Scientific (Pittsburgh, PA, USA). Stock solutions (1000 μg/mL) of Fe, Zn, Se, Cd and yttrium (Y) with certified concentration values for each metal were purchased from SPEX CertiPrep Inc. (Metuchen, NJ, USA). Standard reference material (SRM), Bovine Liver-1577b was purchased from National Institute of Standards and Technology (Gaithersburg, MD, USA).

Human prostate tissue samples were received de-identified (coded) from the National Institute of Health (NIH)-sponsored Prostate Cancer Tissue Resource (www.prostatetissues.org) and kept under −150 °C until use. Studies with human tissues were approved and conducted in accordance with the policies of the Institutional Review Boards of the University of Illinois at Chicago and the Armed Forces Institute of Pathology (Washington, DC). Selected areas from fresh frozen tissue specimens were sliced in three pieces (numbered as 1, 2 and 3) of approximately 10 mm × 5 mm × 2 mm each. Sets of pieces of set 1 (controls), were placed into a vacuum chamber at 50 °C overnight to dry (until a constant weight was obtained), and the sets 2 and 3 were subjected to the standard 10% buffered formalin fixation and paraffin embedding [29] histological process using a tissue processor (Tissue-Tek VIP, Sakura Finetek USA Inc., Torrance, CA). After the paraffin embedding process, tissues were subsequently excised from the blocks with a titanium knife and deparaffinized in xylene at 55 °C for 1 h in the tissue processor (the set 2), or with hexane at 20 °C for 1 week with frequent changes of the solvent in handling-based procedure (the set 3). Xylene was of a grade routinely used for the FFPE process and hexane was of "Optima" grade (Fisher Scientific). Upon deparaffinization, the tissue samples were dried in a vacuum chamber until constant