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Research Paper

Cadmium, arsenic, selenium and iron– Implications for tumor progression in breast cancer

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ABSTRACT

The aim of this study was to determine Cd (cadmium) and As (arsenic) contents in human breast cancer tissues, investigate their interactions with Se (selenium) and Fe (iron), and assess their further implications for tumor progression. Metal contents were determined in 42 tissue sets (tumor and adjacent tissue) collected from 42 women diagnosed with primary breast cancer. Analytical methods included AAS and ICP-MS techniques. Significantly higher contents of Cd ($p = 0.0003$), Se ($p < 0.0001$) and Fe ($p = 0.0441$) whereas significantly lower content of As ($p < 0.0001$) were observed in tumors as compared to adjacent tissues. There was a significant positive correlation between Cd and As contents in tumor tissue. However, only Cd was significantly associated with histological type of tumor, its size, grading and progesterone receptor status. This study support the role of Cd in breast cancer risk and progression. The possible link between As exposure and breast cancer is still not clear.

1. Background

Cadmium (Cd) and arsenic (As) are widely distributed environmental pollutants, classified according to IARC (The International Agency for Research on Cancer) as human carcinogens (Iarc Working Group on the Evaluation of Carcinogenic Risks to Humans, 2012; International Agency on Cancer Research, 1993). Along with the carcinogenic and toxic properties, these heavy metals were shown to activate estrogen receptors in the absence of estradiol and thus they have been implicated in human breast cancer development (Davey et al., 2007; Kortenkamp, 2011). A special attention is paid in this context to Cd which due to its long biological half-life, accumulates in the body posing threat to human health (Luevano and Damodaran, 2014). However, epidemiological studies investigating the relationship between environmental exposure to Cd and risk of breast cancer are not consistent, as shown by recent three meta-analyses (Larsson et al., 2015; Van Maele-Fabry et al., 2016; Wu et al., 2015). Studies investigating urinary Cd concentrations (as a biomarker of long-term Cd exposure) indicate that high Cd exposure may increase the risk of breast cancer (Larsson et al., 2015), whereas no significant association with breast cancer incidence was found when dietary Cd intake was assessed (Van Maele-Fabry et al., 2016; Wu et al., 2015). Overall, the causative relationship seems to depend on several hormone related factors, including menopausal status and hormone receptor status. For example, high Cd intake was associated with increased risk of estrogen positive (but not estrogen negative) breast cancer in postmenopausal Japanese women (Itoh et al., 2014). Direct causative link between breast cancer and the second metalloestrogen As, is not clear, mainly due to scarce epidemiological evidence (Baastrup et al., 2008). Recent case control study conducted among Mexican women shed more light on this association (Lopez-Carrillo et al., 2014). It was observed that it is not As exposure per se but rather individual capacity to methylate inorganic As compounds that directly impacts breast cancer risk. On the other hand, As exerts therapeutic properties and large body of evidence shows that As compounds are able to induce apoptosis in breast cancer cells, indicating for dual role of this metal in breast cancer development (Chow et al., 2004; Wang et al., 2011; Xia et al., 2012). In line with these *in vitro* observations, high environmental exposure to As through drinking water has been correlated recently to decreased breast cancer mortality (Smith et al., 2014).

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Apparently, more studies need to be done to explain the effects of environmental exposure to metalloestrogens on breast cancer risk and identify factors which could affect this relationship. One of the potentially important modifiers is selenium (Se), essential trace element which is able to activate estrogen receptors as well (Stoica et al., 2000) and which was shown to exert anticancer effects *in vitro* in breast cancer cells, as shown already in 1984 (Watrach et al., 1984) and confirmed by later studies (Guo et al., 2015; He et al., 2013). Importantly, epidemiological data suggest that high exposure to Se may decrease breast cancer risk (Cai et al., 2016). Interactions of Se with both metalloestrogens are supported by numerous *in vitro* and *in vivo* observations showing that Se, due to its antioxidant properties, counteracts toxic effects related to Cd and As exposure (reviewed in (Zwolak and Zaporowska, 2012)). Additionally, Se may interact with As more directly, as both metals share metabolic pathways linked to methylation (Sun et al., 2014). Based on mice model, Se was proposed to protect from Cd carcinogenicity in human breast cancer (Schrauzer, 2008), and recent observational study in humans seems to support this hypothesis, showing that breast cancer risk associated with urinary Cd was modified in postmenopausal Chinese women by urinary Se (Wei et al., 2015).

Another element which may potentially modify the effects of exposure to Cd and As, is iron (Fe), the most abundant transition metal in the human organism. Though being essential for cellular processes, Fe is suggested to promote carcinogenesis, when taken in excess and epidemiological data indicate for a significant role of dysregulated iron homeostasis in breast cancer (Marques et al., 2014). Fe may exert toxicity through enhanced oxidative stress, by contributing to the production of highly toxic molecules (hydroxyl radicals and anions) in the presence of hydrogen peroxide (known as Fenton and Haber-Weiss reactions) (Marques et al., 2014). Cd may interact with Fe, by replacing it in protein binding sites and as a consequence, the effects of Cd toxicity largely depend on the general Fe status (Moulis, 2010). Cd affects Fe homeostasis mainly by pathways involving Fe-related proteins, like ferritin, which is an ubiquitous protein responsible for Fe storage and release (Marques et al., 2014). Interestingly, ferritin serum levels were found to be significantly higher in breast cancer women as compared to healthy controls (Jacobs et al., 1976). Possibly, ferritin may also mediate interactions between Fe and second metalloestrogen, As, since methylated As species were shown to cause release of Fe from ferritin (Ahmad et al., 2000).

Studies on the interactions between metals in the carcinogenesis of mammary gland are very limited though substantial evidence has shown that breast cancer tissues cumulate both heavy metals and transition metals (Ionescu et al., 2006; Mohammadi et al., 2014; Strumylaite et al., 2011). Since some metal-metal interactions can either inhibit or promote carcinogenesis, the effect of exposure to metalloestrogens such as Cd and As on breast cancer pathology, may largely depend on trace elements content in the mammary gland (Madden, 2003). The aim of this study was to determine the content of Cd, As, Se and Fe in breast cancer tissues and the adjacent specimens, with further implication for disease progression and tumor characteristics.

2. Methods

2.1. Study group

84 tissue specimens (42 tumor tissues and 42 adjacent tissues) from 42 women diagnosed with primary breast cancer were collected intraoperatively at the Medical University of Gdansk Hospital in the years 2006–2013. Adjacent tissues were taken at least 2 cm from tumor boundary. Matched samples of tumor and adjacent, uninvolved mammary glandular tissue were collected from the opposite quadrants of breast. Histopathological evaluation was performed independently by two pathologists. Tumors containing less than 50% of tumor cells in total cell mass as well as adjacent tissues that contained any tumor cells, were rejected from the analysis. Tissue specimens were stored in the biobank at −70 °C until analyses. All patients signed informed consent and the study was approved by relevant Local Ethics Committees (Independent Ethics Committee at Medical University of Gdansk, resolution No. NKEBN/781/2005, and Ethical Institutional Review Board at the Nofer Institute of Occupational Medicine resolution No. 19/2014). Characteristics of the study groups are presented in Table 1.

Table 1
Study group characteristics.

Variable	
n (%)	42 (100)
Age (years)	61.6 ± 13.5 (32–86)[a]
BMI (kg/m^2)	26.2 ± 4.0 (18–38)[a]
Smoking status, n (%)	
Yes	8 (19.0)
No	33 (78.6)
Unknown	1 (2.4)
Menopausal status, n (%)	
premenopausal	8 (19.0)
postmenopausal	34 (81.0)
Tumor type, n (%)	
IDC	29 (69.0)
ILC	7 (16.7)
Other	6 (14.3)
Tumor stage, n (%)	
T1	22 (52.4)
T2	11 (26.2)
T3	6 (14.3)
T4	3 (7.1)
Grading, n (%)	
G1	2 (4.8)
G2	26 (61.9)
G3	13 (31.0)
unknown	1 (2.4)
Lymph node status, n (%)	
N0	21 (50.0)
N1	14 (33.3)
N2	4 (9.5)
N3	2 (4.8)
Nx	1 (2.4)
ER status, n (%)	
positive	30 (71.4)
negative	11 (26.2)
unknown	1 (2.4)
PR status, n (%)	
positive	30 (71.4)
negative	11 (26.2)
unknown	1 (2.4)
HER2 status, n (%)	
positive	20 (47.6)
negative	20 (47.6)
unknown	1 (2.4)

IDC – invasive ductal carcinoma, ILC – invasive lobular carcinoma, ER – estrogen receptors, PR – progesterone receptors, HER2–human epidermal growth factor receptor 2.
[a] mean ± SD (range).

2.2. Determination of Cd, As, Se and Fe in tissues

Tissue samples were mineralized in a concentrated spectrally pure nitric acid (Tracepur, Merck, Darmstadt, Germany) using closed microwave digestion system (Ertec, Wroclaw, Poland). Cd concentration was determined by AAS (atomic absorption spectrometry) with electrothermal atomization, at a wavelength of 228.8 nm and with a