RESEARCH COMMUNICATION

Association between Glycodelin and Aryl Hydrocarbon Receptor in Iranian Breast Cancer Patients: Impact of Environmental Endocrine Disrupting Chemicals

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Abstract

Breast cancer affects Iranian women one decade younger than their counterparts in other countries and the underlying risk factors have remained controversial. The aryl hydrocarbon receptor (AhR) mediates the effects of many environmental endocrine disruptors and contributes to the many other genes and Gd is an endocrine-regulated glycoprotein which may induce by AhR ligands in endometrium. This study has aimed to compare the interactions between Gd and AhR and other fundamental genes (p53, K-Ras, ER, PgR, AR) between pre and post menopausal Iranian breast cancer patients. To conduct immunohistochemical studies with appropriate monoclonal antibodies, 25 premenopausal invasive ductal carcinomas and 29 postmenopausal invasive ductal carcinomas were selected retrospectively in 2008-2010 from the pathology department of Imam Khomeini hospital complex of Tehran. Higher levels of AhR in epithelial cells of premenopausal patients and breast fibroadenoma emphasized the susceptibility of these cells to environmental induced tumors. Current study demonstrated a significant association between tumoral levels of Gd and AhR (p=0.002) in breast cancers which confirms the preliminary hypothesis about the role of TCDD exposure on Gd biosynthesis and secretion in TCDD-treated endometrial epithelial cells. In summary this study showed the dual prognostic role of Gd especially in premenopausal breast cancer which could be induced by AhR overexpression. Further studies are necessary to find the direct role of breast carcinogens as well as endocrine disrupting chemicals on the differential levels of Gd in breast tumors.

Key words: Breast cancer - AhR - endocrine disruption - Iran

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Introduction

Although the probability of having breast cancer increases with the age in general, this malignancy affects Iranian women at least one decade younger than their counterparts in other countries (Yavari et al., 2005; Hajian-Tilaki et al., 2011). Because of the devastating clinical and psychosocial effects of breast cancer in younger women, identification of environmental risk factors could lead to primary prevention through risk assessment and risk reduction of breast cancer in young patients (Chlebowski, 2000; Lux et al., 2005). An evolving body of literature suggests that breast cancer may have an environmental origin and the particular concern is on hormonally active environmental agents such as dioxin and other persistent compounds that bio-accumulate and magnify within the food chain. Halogenated and nonhalogenated polycyclic aromatic hydrocarbons (HAHs/PAHs), such as polychlorinated dibenzo-p-dioxins and biphenyls and benzo)a)pyrene, have been recognized as significant and widespread environmental contaminants which induce the expression of AhR in target tissues especially in breast (Pliskova et al., 2005). AhR is a ligand-activated transcription factor which mediates the adverse effects of mentioned toxicants in biological organisms (Van der et al., 2009). We have recently reported higher levels of tumoral AhR in young (premenopausal) breast cancer patients (Bidgoli et al., 2010) but further studies are necessary to compare the interaction between AhR levels and endogenous factors between pre and post menopausal breast cancer patients.

Gd is an endocrine-regulated glycoprotein that has significant effects on immune cells, apoptosis, reproduction, cell adhesion, differentiation and cancer. In endocrine-related hormone-responsive tumours,

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Gd-induced differentiation is related to the decreased expression of oncogenes and an increased expression of tumour suppressor genes, emphasizing the tumour suppressor nature of Gd in hormone responsive tumors (Seppala et al., 2009). The significance of Gd expression in the clinical studies of breast cancer patients shows that Gd expression varies according to the clinical stage (Jeschke et al., 2005). Although there are many studies regarding the role of Gd in breast cancer prognosis, its differential prognostic role in different breast cancer cells, its specific clinicipathological significance in young premenopausal breast cancer patients as well as its interaction with AhR have not identified yet.

We have proposed in this study that the interactions between Gd and AhR could be considered as a responsible factor for early onset of breast cancer. This study is concerned to the differential expression of Gd in pre and post menopausal breast cancer and its interaction with AhR and fundamental breast cancer genes (p53 and K-ras) as well as sex steroid receptors (ER alpha and PgR) using immunohistochemical methods which are valid methods for determination of markers in reproductive tissues (Scholz et al., 2009).

Materials and Methods

Fifty four patients (29 postmenopausal breast invasive ductal carcinoma and 25 pre menopausal breast invasive ductal carcinoma) whose tissues contained necessary adjacent normal tissues were selected retrospectively for this study. These patients underwent surgery in Imam Khomeini University Hospital complex during the years 2008–2010 therefore their demographic information including age at diagnosis, marital status, history of childbirth, history of lactation, menopausal status, occupational situation, history of breast tumors, familial history of breast cancer or other malignancies, smoking and alcohol consumption were collected by filling out the questionnaires from their documents or during performing an interview with accessible patients. Patients were excluded if they were habitual heavy drinker, IV drug abuser or having any evidence of underlying endocrine disorder in their medical history. As we decided to analysis the AhR expression in sporadic breast cancer patients, cases with familial history of breast cancer or patients who showed BRCA1 and BRCA2 expression in their tumoral cells were excluded from present study. Tumor anatomical location and tumor size were recorded from the general reports of patients. Breast surgical samples were scored for histological grade, pathological stage, lymphatic invasion, neural invasion, vascular invasion, secondary organ metastasis, nuclear grade, tumor calcification and existence of fibrocystic tissues by two pathologists who were blinded to the IHC scores and clinical features of patients.

As previously described(Bidgoli et al., 2010), dewaxed and rehydrated tissue sections were subjected to antigen retrieval using microwave oven and boiling citrate buffer (pH=6.0). Endogenous peroxidase activity and nonspecific binding sites were blocked by incubating sections by 0.3% hydrogen peroxide in methanol for 30 min. and 3% BSA for 60 min, respectively. Sections were then incubated 30 minutes at Room Temperature with AhR mouse monoclonal antibody (clone PRT9, abcam), Gd (clone 001-13,abcam), P53(clone DO7, Dakocytomaion), k-RAS (clone 234-4.2, abcam), ER alpha (Clone 105, Dakocytomation), PgR (Clone 636, Dakocytomation) that recognize the nuclear and cytoplasmic expression of human proteins in breast tissues. The results were visualized using the envision system (Dakocytomation) based on the manufacturer's instruction with necessary modifications. Sections were also counterstained with Meyer's haematoxyline. In each series, a section in which incubation with the primary antibody was omitted used as negative control. The ideal staining conditions were established in our preliminary experiments. Staining was considered negative only after careful examination of the entire tissue section.

Immunohistochemistry is an indispensable research and diagnostic tool used to assess the presence or absence of molecular tumor markers on paraffin-embedded tissue. Tumor positivity for a given marker is frequently evaluated using predetermined cutoffs such as 10% (≤10% tumor cells staining=negative, >10%=positive). The employment of categorical scoring systems is motivated by the ease of interpretation of positive tissue by pathologists and is further supported by substantial inter observer agreement(Zlobec et al., 2006). Semiquantization of the intensity and number of positive breast cells was performed differentially in malignant, cells by two independent pathologists in this study. Other than invasive carcinoma cells other cells including stromal, lymphatic, vascular, myoepithelial and adipose was scored semiquantatively. For all mentioned cells, staining intensity and pattern were evaluated using the semi-quantitative scale of 0–3+. Most of samples contained adjacent benign epithelium therefore the score subtracts the score on the benign cells from that on the tumor cells. If benign epithelial cells were not present in the section, the non-normalized score on the tumor was used (Zlobec et al., 2006).

Each cell type in surgical samples was then classified into four categories based on the expression patterns of markers. Based on quantization of the intensity and number of positive cytoplasms or nuclei, each cell type was scored. In cases in which the observers disagreed in final score, the immunohistochemical scoring was repeated to agree on same scoring by both observers. Adding the two scores gives a maximum score of 5. All cells in each tissue section were considered by 100 x, 250x and 400 x. The parafinazed tissues came from archives of department of Pathology, Imam Khomeini Medical University Complex.

For analyses, values were expressed as percent per population or as the mean±standard deviation (SD). To assess the association between expressions of markers

and clinicopathological data nonparametric chi-square test was used. Relative risks and Odds ratios were calculated by Cochran's and Mantel Haenszel statistics using SPSS 16.Probability values of <0.05 and Odds ratios>1 were considered significant.

Results

The clinicopathological features of pre and post menopausal breast cancer patients of this study have been described before (Bidgoli et al., 2010). Except higher risk of vascular invasion (OR=1.96, CI 95% 0.523-7.370)) in premenopausal patients other pathological factors didn't show any clinical importance. Out of different breast cancer cell types Gd was found in epithelial and lymphatic cells only. Figure 1 shows the differential expression of Gd in 38% (n=11) of epithelial cells of breast cancer patients whereas it was not found in adjacent stromal cells of malignant tumors. Lymphatic expression of Gd was detectable in 24% (n=6) of patients too. Clinicopathological significance of Gd was evaluated in malignant epithelial cells and lymphatic cells separately. Table 1 shows the clinicopathological significance of Gd in malignant cells of breast invasive ductal carcinoma. The mean size of Gd positive tumors were 5.58±4.8 cm whereas the mean size of Gd negatives were 3.62±2.11 (p=0.019). Clinicopathological significance of Gd was evaluated in lymphatic cells separately. Higher levels of Gd in lymphatic cells which were associated with higher risk of lymphatic invasion (OR=1.179 CI 95% 1.02-1.36). Other factors were not significantly contributed to the lymphatic levels of GD.

Close association was observed between tumoral levels of Gd and AhR (P=0.002). Higher risk of Gd expression was observed in AhR (+) tumors (OR= 5.542, CI 95% 1.34-22.771). Significant association between tumoral Gd and AhR expression in premenopausal breast cancer patients (p=0.036) was observed. Four possible immunophenotypes were calculated in pre and post menopausal breast cancer patients. The immunephenotype (AhR+/ Gd+) was more common in young/premenopausal breast cancer patients (35.5% vs. 20%, p=0.035).

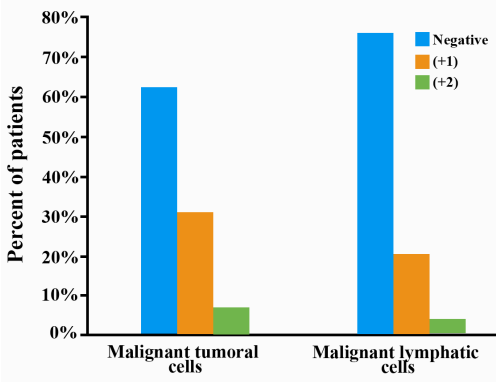

Figure 1. Differential Expression of Gd in Malignant Epithelial and Lymphatic Cells of Breast Invasive Ductal Carcinoma

Table 1. Clinicopathological Significance of Gd in Breast Invasive Ductal Carcinomas

Characteristics	Cases	Controls	P-value
Vascular Invasion			
Positive	21(40.4%)	11(21.1%)	0.35
Negative	16(30.8%)	4(7.7%)	
Neural Invasion			
Positive	10(19.2%)	8(15.4%)	0.17
Negative	27(51.9%)	7(13.5%)	
Lymphatic Invasion			
Positive	22(42.3%)	12(23.07%)	0.11
Negative	15(28.9%)	3(5.77%)	
Histological Grade			
I	0(0%)	0(0%)	0.56
II	3(13.7%)	1(4.55%)	
III 11(50%)	7(31.8%)		
Fibrocystic Tissues			
Positive	28(53.9%)	9(17.3%)	0.35
Negative	9(17.3%)	6(11.55%)	
Calcification			
Positive	31(59.6%)	12(23.1%)	0.94
Negative	6(11.53%)	3(5.77%)	
Age (yrs)	47.8±14.3	45±10.6	0.20
Tumor Size (cm)	3.62±2.11	5.58±4.8	0.01*

Table 2. Differential Expression of Gd, ER, PR, p53 and K-ras in Premenopausal Cancers (n=25)

	0	1+	2+	3+	4+	5+
AhR	4	8	5	3	3	2
ER	17	3	2	2	1	0
PgR	15	4	2	3	0	1
P53	17	4	2	2	0	0
k-RAS	10	8	4	3	0	0

Table 2 compares the differential levels of Gd, ER, PR, p53 & K-ras in pre and post menopausal breast cancer patients. Significant associations between tumoral Gd and PgR, ER, P53 and K-ras were not detected in total group of breast cancer patients (P>0.05) but a close correlation between tumoral p53 and Gd was detectable in premenopausal breast cancer patients. The immunophenotype (p53+/Gd+) was more common in young/premenopausal breast cancer patients (p=0.044).

Discussion

Exposure to endocrine disruptors which are AhR ligands induce abnormal genital tract development and decreases fertility in rodent species(Hurst et al., 1998; Bofinger et al., 2001). Dioxin, TCDD and similar organic toxicants might play direct mechanistic roles in the etiopathogenesis of endometriosis and female infertility by increasing Gd production but the importance of this association in breast cancer was demonstrated for the first time in this research. Current study demonstrated a significant association between tumoral levels of Gd and AhR (p=0.002) in breast cancer which confirms the preliminary hypothesis about the role of TCDD exposure on Gd biosynthesis and secretion in TCDD-

treated endometrial epithelial cells (Mueller MD et al., 2005). Because the action of dioxin is mediated by the aryl hydrocarbon receptor (AhR), we ascertained that AhR levels is closely associated with Gd in breast cancer which may be dose related too.

Among different immunophenotypes which were assessed in pre and post menopausal breast cancer patients AhR+/ Gd+ immunophenotype was more common in premenopausal patients (35.5% vs. 20%, p=0.035). Recent studies have showed a dual action for AhR ligands on the breast, with different effects in pre and postmenopausal women. AhR ligands may increase the risk of breast cancer in premenopausal women (Band et al., 2002) whereas increased risk of postmenopausal breast cancer was seen in women with higher urinary excretion of both estrogens and androgens (Onland-Moret et al., 2003). This finding may strengthen the hypothesis on environmental basis of sporadic premenopausal breast cancer.

We showed also higher risk of lymphatic invasion (OR=1.179 CI 95% 1.02-1.36) in women who expressed Gd in their lymphatic cells. Higher tumoral levels of AhR were also contributed to the larger tumor sizes)(5.58±4.8 cm vs. 3.62±2.11 (p=0.019)). This may emphasize the importance of exposure to endocrine disruptors on AhR and Gd levels and tumor prognosis. Although one recent study showed the correlation of Gd with better clinical outcome in sporadic breast cancer without familial history (Hautala et al., 2011), we showed the differential prognostic role of Gd in lymphatic and tumoral cells for the first time in this study.

Premature or inappropriate endometrial expression of Gd also has been documented in women using oral contraceptives and progestin-releasing intrauterine devices (Mueller MD et al., 2005). Present findings didn't show any direct association between Gd and of sex steroid receptors. In fact one of the main causes of AhR overexpression in MCF breast cancer cells is the loss of ER alpha functions. This phenomenon is likely to be based on the mutually antagonistic relationship between ER and AhR (Wong et al., 2009). This event is enough critical to suggest AhR as a potential drug target for the treatment of ER negative breast cancer (Zhang et al., 2009). We have observed the same pattern for the first time in a clinical setting in young Iranian breast cancer patients. Premenopausal overexpression of AhR was suggested as a biomarker of exposure to endocrine disrupters and promoted the hypothesis that the early incidence of breast cancer in Iran could be environmental based (Bidgoli et al., 2010). Now the close association between Gd and AhR shows clearly the indirect role of xenoestrogens as endocrine disrupting chemicals which may induce the endogenous levels of Gd which may reduce the prognosis of breast cancer.

In ovaries of rats treated with TCDD (AhR ligand), 19 genes of known function were found to be up-regulated, while 31 ovarian genes were found to be down-regulated)>or=1.5-fold (p<or=0.05)) when compared to controls(Zhang et al., 2009). The higher prevalence of Gd+/p53+immunophenotype in pre- menopausal breast cancer confirms the possible role of Gd on up- regulation of p53. These results strongly suggest that Gd may acts as a tumor suppressor in breast cancer. Although these finding is not compatible with other findings of this study but it is compatible with the observations that certain types of Gd-expressing ovarian and breast cancers have a more favorable prognosis compared to Gd non-expressing tumors (Koistinen et al., 2009). This research has therefore introduced a dual role and complicated mechanism for Gd to control breast cancer cell growth.

In summary this study has showed the dual prognostic role of Gd especially in premenopausal breast cancer which could be induced AhR overexpression by exposing to AhR ligands. Further studies are necessary to find direct role of breast carcinogens as well as endocrine disrupting chemicals on the differential levels of Gd in breast tumors, its interaction with hormonal factors and tumor prognostic factors.

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