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BRIEF COMMUNICATION

Aromatase, CYP1B1 and Fatty Acid Synthase Expression in Breast Tumors of BRCA1 Mutation Carriers

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Abstract Numerous experimental evidence suggest that BRCA1-associated breast carcinomas may have distinct endocrine and metabolic features, however these peculiarities are poorly evaluated in clinical settings. Here we comparatively analyzed for the first time aromatase, estrogen 4-hydroxylase (CYP1B1) and fatty acid synthase immunohistochemical expression in breast tumors obtained from 12 BRCA1 mutations carriers and 22 non-carriers. Aromatase expression was higher in mutation carriers than in sporadic cases ($p=0.04$), which confirms the earlier results obtained in cell lines with down-regulated wild-type BRCA1 and corroborates the usage of aromatase inhibitors in such patients. No differences between study groups were found in the expression of CYP1B1 and fatty acid synthase, which does not, however, mitigate the need of further search for manifestations of the excessive genotoxic effects of estrogens and for increased lipogenesis in BRCA1 mutations carriers.

Keywords Breast cancer · BRCA1 · Hormonal-metabolic factors · Aromatase · Catechol estrogens · Lipogenesis

In the carriers of BCRA1 gene mutations, mammary cancer represents a special form of the disease as suggested by at least two of its features: (a) a relatively early onset because of severe impairments in DNA repair [1] and (b) specific endocrine-metabolic features [2–4].

Indeed, BRCA1 silencing in MCF-7 cells is associated with increased aromatase activity and gene expression [5, 6] and with the stimulation of the transcriptional activity of ER-α [4]. At the same time, breast cancer in carriers of mutated BCRA1 features a propensity to the estrogen-negative (and often even triple-negative) receptor phenotype and higher grade of malignancy [7, 8]. In contrast to the classic estrogen estradiol which stimulates expression of wild-type BRCA1 gene, 4-hydroxyestradiol (the most carcinogenic and genotoxic catecholestrogen generated with CYP1B1 involvement) decreases its copy number [9], and thus eliminates suppressor functions of this gene. In addition, deleting the murine analogue of BRCA1 is associated with increased spontaneous mammary tumors incidence and enhanced reactive oxygen species generation and oxidative stress-caused lethality [10]. Resting on these and other data, an increased susceptibility of the carriers of BCRA1 mutations to the genotoxic type of estrogen-induced cancer is suggested [11] and warrants systematic assessment.

Other consequences of mutant BCRA1 carrying may include disturbances in insulin and/or insulin-like growth factor-dependent processes and in lipogenesis. In MCF-7 cells, this is manifested as the activation of IGF1 receptor gene promoter [12] and a marked increase in fatty acids synthesis upon down-regulation of BRCA1 expression [13].

In accordance with said above, the present study was aimed to compare aromatase, estrogen 4-hydroxylase (CYP1B1) and fatty acid synthase expression in breast cancers from women with or without BCRA1 mutations.

BRCA1 mutations (5382insC being the most frequent among them) were detected as described earlier [14]. Breast

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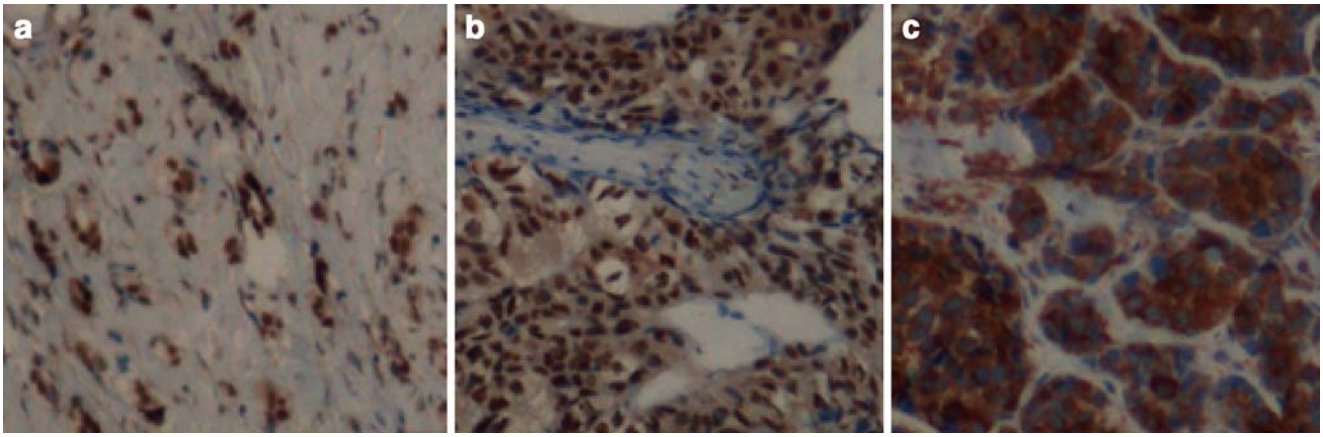

Fig. 1 The examples of immunohistochemical staining of aromatase (**a**, score 2), CYP1B1 (**b**, score 3) and fatty acid synthase (**c**, score 4) in breast cancer tissue

carcinomas randomly selected for this study included 12 tumors from mutant BRCA1 carriers (mean age 43.3± 2.3 years) and 22 tumors from patients who were found to be free of BRCA1 mutations (mean age 47.4±2.0 years). In both groups mostly invasive ductal carcinomas with moderate or high grade of malignancy were revealed. Four-micron sections of paraffin-embedded tumor blocks were deparaffinized in xylene, and stained for the expression of aromatase, CYP1B1, and fatty acid synthase, FASN (Fig. 1). For this purpose we used mouse monoclonal antibody (AbD Serotec, anti-aromatase MCA2077S), rabbit polyclonal antibody (Abcam Inc, anti-CYP1B1 ab33586), and mouse monoclonal antibody (Abcam Inc, anti-FASN ab54654). Briefly, the sections were treated with 3% H_2O_2 for 5 min to block endogenous peroxidases, and then incubated overnight at +4°C with the primary antibody at a 1:200 (aromatase) and 1:500 (FASN) dilutions, or for 30 min at room temperature and at a 1:1500 dilution (CYP1B1). No first antibody had been used in control slides. After several washes, the sections were processed for half an hour at room temperature, with mouse or rabbit EnVision$^+$ system ("Dako", Denmark) respectively, and then stained with 3,3′-diaminobenzidine (Vector Laboratories) for 5 min. Counterstaining with hematoxylin was performed for 2 min. Four to five different fields from each section were analyzed independently by two co-authors (K.P. and L.B.) and evaluated semi-quantitatively according to proportion of positive staining cells using the following scoring systems: 0 (no staining), 1 (weak), 2 (moderate), and 3 (strong) for aromatase (cytoplasmic staining) and 0 (negative), 1 (weak), 2 (moderate), 3 (strong), and 4 (very strong) for CYP1B1 (nuclear staining) and fatty acid synthase (cytoplasmic staining). All results were worked up by one way analysis of variance using SigmaPlot program kit. Data comparison was performed using unpaired Student's *t* test as well as by hi-square test. Data are presented as mean ± SEM, and statistical significance is defined as $p<0.05$. Correlation coefficients were calculated according to Spearman. This investigation was approved by Local Ethic Committee.

The results of the analysis are summarized up in Table 1. Only aromatase activity was found to be increased in breast tumor tissues of mutant BCRA1 carriers (t 2,11, p 0.04; hi-square 3,87, p 0.05), whereas no significant differences between study groups were found in the expression levels of CYP1B1 and fatty acid synthase. Moreover, the latter showed a moderate trend towards greater expression in the patients found free of BRCA1 mutations. Rank correlation analysis showed a negative correlation between aromatase and estrogen receptor expression levels (−0.61, $p=0.04$) and between CYP1B1 and progesterone receptor expression levels (−0.62, $p=0.03$) in the tumors of mutant BRCA1 carriers and a positive correlation between aromatase and fatty acid synthase expression levels (+0.47, $p=0.02$) in the patients free of BRCA1 mutations.

Table 1 Average data (M±SE) on immunohistochemical evaluation of aromatase, CYP1B1 and fatty acid synthase expression in breast cancer tissue: comparison of carriers and non-carriers of BRCA1 mutations

Group	Number of cases	Immunohistochemical score (cond.un.)		
		Aromatase	CYP1B1	Fatty acid synthase
With BRCA1 mutations	12	1,33±0,37*	2,08±0,45	2,66±0,35
Without BRCA1 mutations	22	0,45±0,16*	1,86±0,25	3,00±0,23

*$p=0,04$

In summary, the first clinically based comparison of breast cancers in patients who bear BRCA1 mutations or are free of them has confirmed the experimental data that suggest the activation of the key enzyme of estrogen biosynthesis, i.e. aromatase, upon 'transition' from wild type BRCA1 to its mutated forms [5, 6], but presented no signs of increased expression of estrogen 4-hydroxylase and fatty acid synthase under the same conditions. However, the search for such signs in BRCA1-related breast cancer should not be limited by studies of tumor tissues only and may include, in particular, the assessment of urinary excretion of certain catecholestrogen fractions [15] and other indices of the genotoxic effects of estrogens [16]. With regard to lipogenesis, it is possible that the upstream deficiencies (e.g., involving acetyl coenzyme A carboxylase-α) resulting from BRCA1 mutations are more important than the downstream ones (e.g., fatty acid synthase, FASN) [13, 17] because FASN is overexpressed in most breast neoplasms [17, 18]. Finally, although aromatase activation found in the present work is still to be confirmed using other assays (e.g. radiometrical, CYP19 mRNA expression, etc.) and the highly recommended monoclonal antibody #677 for immunohistochemistry [19], the conformance of the present clinical data with published experimental results [5, 6] corroborates the appropriateness of aromatase inhibitors in therapy of the breast cancers featuring BRCA1 mutations [20, 21].

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