doi:10.4149/neo_2010_04_325

CCND1 and *ZNF217* gene amplification is equally frequent in *BRCA1* and *BRCA2* associated and non-*BRCA* breast cancer

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Received September 9, 2009

Breast cancer associated with *BRCA1* and *BRCA2* gene mutations differs from non-*BRCA* tumors in several respects. We determined whether there was any difference in *CCND1* (11q13) and *ZNF217* (20q13) gene amplification with respect to *BRCA* status. Of 40 breast cancer samples examined, 15 and 9 were from *BRCA1* and *BRCA2* mutation carriers, respectively, and 16 from patients without mutation. Fluorescence in situ hybridization showed that eight tumors exhibited *CCND1* amplification (20%; 3 *BRCA1*, 3 *BRCA2,* 2 non-*BRCA*). *ZNF217* amplification was observed in three of 38 cases (8%; 2 *BRCA1*, 1 non-*BRCA*). There was no significant difference in *CCND1* and *ZNF217* amplification between *BRCA1, BRCA2* and non-*BRCA* tumors. *CCND1* amplification was associated with decreased disease-free (P = 0.045) and overall survival (P = 0.015). *BRCA1* tumors with *CCND1* amplification were estrogen receptor negative, in contrast to *CCND1* amplified *BRCA2* and non-*BRCA* tumors, suggesting that concurrent *CCND1* amplification and estrogen and progesterone receptor negativity may predict germline *BRCA1* gene mutation. All *ZNF217* amplified tumors were of the medullary histological type (P = 0.002). There was no statistical correlation between *CCND1* and *ZNF217* amplification and estrogen receptor, progesterone receptor, and ERBB2 expression and TNM classification. *CCND1* amplification did not correlate with EGFR expression.

Key words: CCND1 gene amplification; ZNF217 gene amplification; BRCA1 gene; BRCA2 gene; fluorescence in situ hybridization, breast cancer

Genome alterations and aneuploidy are frequently found in breast cancer. Indeed, several chromosomal regions of amplification have been identified and characterized, the most frequent being 8p12, 8p24, 11p13 and 17q12 [1]. Each of these is found in 10 to 25% of cases [1]. Amplification at 20q13 is slightly less frequent and occurs in around 5 to 12% of cases [2]. Several genes appear to be involved in the 8p12 amplification, including the *RAB11FIP1* and *FGFRI* genes, while the *MYC* gene is involved in 8q24 amplification, the *CCND1* gene in 11q13, the *ERBB2* gene in 17q12, and the *MYBL2, ZNF217* and *STK6* genes in 20q13 amplification [reviewed in 2]. Amplification of *ERBB2* is a factor of poor prognosis and amplification of *CCND1* is more frequent in tumors with positive estrogen receptor (ER) status. Aneuploidy and aggressive behavior are often associated with 20q13 amplification [3, 4]. Women with germline mutations in the *BRCA1* and *BRCA2* tumor suppressor genes are known to be predisposed to breast and ovarian cancer [5]. Breast cancer associated with *BRCA1* and *BRCA2* gene mutations differs from other breast cancer cases in several aspects. Most *BRCA1* carcinomas have a basal cell phenotype, a subtype of high-grade, highly proliferating, triple-negative breast cancer (i.e. with negative ER, progesterone receptor (PR), and ERBB2 (v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog) status), characterized by the expression of basal or myoepithelial markers such as basal keratins, P-cadherin and epidermal growth factor receptor (EGFR) [6, 7]. This phenotype is rarely found in *BRCA2* carcinomas, which are of a higher grade than sporadic age-matched controls but tend to be ER and PR positive [7–10]. An infrequent loss of PML protein expression and a more frequent aberrant reduction in ATM protein expression have been described among *BRCA1* tumors [11, 12]. In addition, the expression of the cell-cycle proteins cyclin A, B1 and E has

been associated with the *BRCA1* phenotype, whereas p27 expression has been associated with *BRCA2* carcinomas [7]. In almost all studies, no significant difference has been reported in p53 status between *BRCA1, BRCA2* and sporadic tumors, *BRCA1* and *BRCA2* tumors being predominantly p53 positive [13]. Similarly, no significant difference has been found in the frequency of cathepsin D, bcl-2, p21, brc-2, proliferating cell nuclear antigen (PCNA), cyclin E, catepsin E, E-cadherin and beta-catenin expression [14–16].

While two studies have been published that determine *CCND1* gene amplification with respect to *BRCA* status, their results are contradictory [16, 17]. Amplification of the *ZNF217* gene in association with *BRCA1* and *BRCA2* gene mutations has not yet been studied.

The aim of this study was to determine whether there is any difference in *CCND1* (11q13) and *ZNF217* (20q13) gene amplification in breast cancer of women with and without the *BRCA1* or *BRCA2* gene mutation and whether *CCND1* and *ZNF217* gene amplification correlates with any clinical or histopathological characteristics.

Materials and methods

The study population comprised 40 breast cancer samples from 40 unrelated female patients, of whom 15 were *BRCA1* germline mutation carriers, 9 were *BRCA2* germline mutation carriers and 11 were patients without a *BRCA1* or *BRCA2* germline mutation. Formalin fixed paraffin-embedded tumor samples were studied. Clinical data and histological findings were found in the health care documentation. Unfortunately, these data were not available in all the patients and in some patients, there was not enough material to perform immunohistochemical analyses.

The tumors were classified into the following five histological categories: ductal carcinoma grade 1 (low grade), 2 (medium grade) and 3 (high grade), lobular carcinoma and mucinous carcinoma. The TNM (Tumor, Node, Metastasis) stage was classified according to the 6th edition of "TNM classification of malignant tumours" [18].

Indirect immunohistochemistry was used to evaluate ER, PR, PCNA, ERBB2 and EGFR expression. Prior to immunostaining, heat-induced antigen retrieval was performed by treatment in a microwave oven. Mouse monoclonal antibodies against estrogen receptor α (clone 1D5; DakoCytomation, Coppenhagen, Denmark), progesterone receptor (DakoCytomation, Denmark), PCNA (clone PC10, DakoCytomation, Denmark) and EGFR (NeoMarkers, California, USA) were used with the EnVision™ amplification system (DakoCytomation, Denmark). For ER, PR and PCNA, samples with less than 10% positive cells were considered negative (0), those with 10–25% of positive cells as weakly positive (1), those with 25–75% of positive cells as moderately positive (2) and samples with 75–100% of positive cells as strongly positive (3). Membrane and/or cytoplasmic expression of EGFR was evaluated semiquantitatively as negative (0), slight (1+), moderate (2+) and intense (3+) [19]. ERBB-2 status was evaluated through immunohistochemistry using the 0–3+ score as suggested by the HercepTest kit scoring guidelines (DakoCytomation, Denmark).

Formalin-fixed, paraffin-embedded tumor tissue was cut into 3 μm slices, placed on SuperFrost Plus slides and dried at 56°C overnight for fluorescent *in situ* hybridization (FISH). The slides were deparaffinized using the Paraffin Pretreatment Reagent Kit of Vysis Inc. (Downers Grove, Illinois, USA). Gene amplification was primarily analyzed using commercial probes supplied by Vysis Inc. (Downers Grove, USA). The directly labeled specific LSI Cyclin D1 (11q13; Spectrum Orange-labeled) and centromeric CEP11 (11p11.11-q11; Spectrum Green-labeled) probes were used for *CCND1* amplification. *ZNF217* amplification was detected using the LSI 20q13 (20q13.2; Spectrum Orange-labeled) and telomeric TelVysion 20p (20ptel; Spectrum Green-labeled) probes or the centromeric Satellite Enumeration Probe SE 20 (D20Z1; Spectrum Green-labeled) from Kreatech (Amsterdam, the Netherlands). All analyses were performed according to the manufacturers´ instructions. In each case, we evaluated 50–100 non-overlapping interphase nuclei with preserved morphology. The slides were viewed with an Olympus BX60 fluorescence microscope with three one-band pass filters and the images captured with a CCD camera, whereupon the images were filtered and processed using the Lucia software (Laboratory Imaging Ltd., Prague, Czech Republic). The copy number of the target genes and internal control for each cell was determined under 1 500x magnification. The sample was determined as carrying an amplification of *CCND1* or *ZNF217* gene if the number of gene probe signals divided by the number of centromere or telomere signals, respectively, was ≥1.5 [20] (Figure No. 1). The study was undertaken blind, the laboratory staff undertaking the FISH analysis (DC, AB) was not aware of the *BRCA* status, clinical or histopathological characteristics of the patients involved.

Statistical analysis: Fisher´s exact test was used to correlate gene amplification with *BRCA* status, histological grade, ER, PR, ERBB2, EGFR expression and TNM classification. Correlation with the age of patients was tested using the two-sample t–test. Disease-free and overall survival curves were derived from Kaplan-Meyer estimates and compared using the long-rank test. A P value (two sided) of <0.05 was considered as significant.

Results

CCND1 amplification was found in eight of the 40 samples (20%) and *ZNF217* amplification in three of 38 samples (8%). The amplification rates of the *CCND1* and *ZNF217* genes in the breast cancer samples in relation to *BRCA1* and *BRCA2* gene mutation status and other patient characteristics are summarized in Table 1.

No significant difference was observed in *CCND1* amplification between the *BRCA1, BRCA2* and non-*BRCA* groups (P

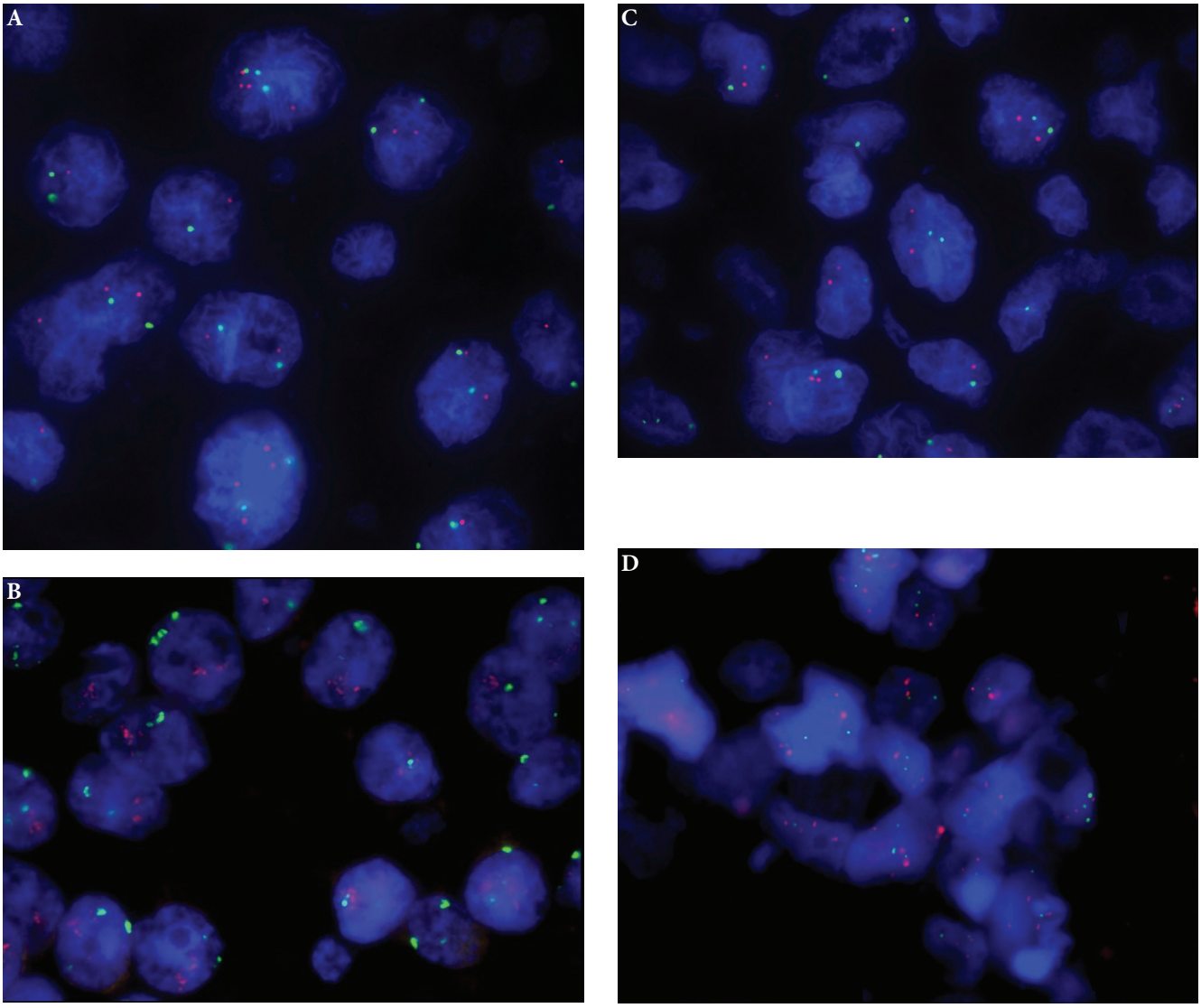

Figure No. 1 Amplification of the *CCND1* and *ZNF217* genes using the FISH method. *CCND1*: **A, normal; B, amplification;** *ZNF217*: **C, normal; D, amplification.**

= 0.464; *BRCA1* versus non-*BRCA*, P = 0.654; *BRCA1* versus *BRCA2,* P = 0.312; *BRCA2* versus non-*BRCA,* P = 0.635). Similarly, no significant correlation was observed between *CCND1* amplification and age of patients (P = 0.763), histological typing (P = 0.160), ER (P = 0.915), PR (P = 0.854), PCNA (P = 0.121), ERBB2 (P = 0.871), and EGFR expression (P = 1.000) or with TNM classification (P = 0.905, 0.206 and 0.100, respectively, for pT, pN and pM). *CCND1* amplification did show a significant correlation with decreased overall survival (P = 0.015). In *CCND1*-amplified cancer patients, the average overall survival time was 98 months (95% CI: 43–153 months) and median survival time 69 months (95% CI: 56–82 months), as opposed to non-*CCND1*-amplified cancer patients, where the average overall survival time was 181 months (95% CI: 148–213 months). *CCND1* amplification was also significantly correlated with decreased disease-free survival (DFS) (P = 0.045). In *CCND1*-amplified cancer patients, the average DFS was 91 months (95% CI: 57–125 months) and median DFS 120 months (95% CI: 20-220 months), as opposed to non-*CCND1* amplified patients where average DFS was 199 months (95% CI: 175–222 months). No correlation was observed between *CCND1* amplification and ER and PR status. Although the number of samples was too low to calculate statistical significance, all *BRCA1* patients with *CCND1* amplified tumors were ER and PR negative, whereas all *BRCA2* and non-*BRCA* patients with *CCND1* amplified tumors were ER and PR positive.

As regards *ZNF217* amplification, no significant differences were observed between the *BRCA1, BRCA2* and non-*BRCA*

Table 1. Results of *CCND1* and *ZNF217* gene amplification analysis of breast cancer samples from A) *BRCA1* gene mutation carriers, B) *BRCA2* gene mutation carriers and C) patients with neither *BRCA1* nor *BRCA2* gene mutations, with relevant clinical and histopathological characteristics (n = 40). The figures in red represent samples determined as carrying an amplification (signal ≥ 1.5).

A) *BRCA1* gene mutation carriers

Pt. No.	*Age dg.*	*gen/ cenCCND1*	*gen/tel ZNF217*	*Histol.*	*ER*	*PR*	*ERBB2 (IHC)*	*EGFR*	*pT*	*pN*	*pM*	*DFS (mth)*	*OS (mth)*
2.	52	1.84	1.20	3	0	0	1	1	1	0	0	56	56
3.	49	2.07	1.4	3	0	2	0	0	n.a.	n.a.	n.a.	125	125
4.	49	1.22	1.61	M	0	0	0	n.a.	2	0	0	90	90
5.	49	1.71	1.31	3	0	0	2	0	2	0	0	n.a.	57
6.	46	1.27	1.48	2	0	2	0	1	1	0	0	85	85
7.	42	1.46	1.33	2	0	2	1	0	n.a.	n.a.	n.a.	n.a.	n.a.
8.	51	1.28	1.26	3	2	3	1	0	n.a.	n.a.	n.a.	n.a.	170
9.	34	1.20	1.29	3	n.a.	n.a.	n.a.	n.a.	2	1	0	216	216
10.	55	1.36	1.38	2	2	0	n.a.	n.a.	1	1	0	60	60
11.	38	1.26	1.53	M	0	0	n.a.	n.a.	2	0	0	84	84
12.	63	1.22	1.43	3	0	0	1	2	1	1	0	n.a.	88
13.	39	1.37	1.18	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
14.	38	1.41	1.37	3	0	0	1	0	4	2	1	4	12
15.	37	1.22	1.18	3	1	1	n.a.	n.a.	n.a.	n.a.	n.a.	123	123

B) *BRCA2* gene mutation carriers

Pt. No.	*Age dg.*	*gen/ cenCCND1*	*gen/tel ZNF217*	*Histol.*	*ER*	*PR*	*ERBB2 (IHC)*	*EGFR*	*pT*	*pN*	*pM*	*DFS (mth)*	*OS (mth)*
16.	31	1.30	1.46	3	3	1	2	0	4	1	0	25	38
17.	37	3.67	1.41	3	2	1	2	0	2	1	0	59	58
18.	45	1.05	1.29	2	3	3	2	0	n.a.	n.a.	n.a.	n.a.	n.a.
19.	44	2.10	1.18	L	n.a.	n.a.	1	0	1	0	0	38	69
20.	35	1.26	1.42	3	3	2	0	0	3	0	0	93	93
21.	47	1.20	1.35	2	0	1	1	0	1	0	0	75	75
22.	45	1.45	1.16	3	2	2	2	0	2	0	0	87	87
23.	40	1.30	1.48	3	0	0	3	n.a.	2	0	0	28	28
24.	31	1.95	1.21	2	1	2	0	n.a.	2	2	2	120	180

C) Patients with neither a *BRCA1* nor *BRCA2* gene mutation

Pt. No.	*Age dg.*	*gen/ cenCCND1*	*gen/tel ZNF217*	*Histol.*	*ER*	*PR*	*ERBB2 (IHC)*	*EGFR*	*pT*	*pN*	*pM*	*DFS (mth)*	*OS (mth)*
25.	36	1.15	1.05	CS	n.a.	n.a.	n.a.	n.a.	4	x	0	n.a.	n.a.
26.	22	1.25	1.14	2	0	0	3	1	2	0	0	65	65
27.	33	1.08	1.06	2	2	2	2	0	2	0	0	75	75
28.	56	1.45	1.18	L	3	3	1	0	2	1	0	100	100
29.	35	1.18	1.14	3	0	0	2	2	1	0	0	20	20
30.	39	1.59	1.38	1	2	0	2	0	2	0	0	49	49
31.	63	1.21	1.20	3	n.a.	n.a.	0	n.a.	2	1	0	66	66
32.	38	1.23	1.58	M	0	0	0	n.a.	2	1	0	72	72
33.	42	1.18	1.12	M	n.a.	n.a.	n.a.	n.a.	2	0	0	192	192
34.	53	2.56	1.35	L	3	0	0	n.a.	1	3	1	0	22
35.	63	1.15	1.41	1	n.a.	n.a.	n.a.	n.a.	2	0	0	72	72
36.	63	1.46	1.43	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
37.	31	1.27	1.25	2	0	1	1	0	3	1	0	50	50
38.	64	1.35	-	2	1	0	0	0	2	0	0	82	82
39.	53	1.44	-	3	1	3	0	1	1	0	0	160	160
40.	57	1.19	1.29	L	0	0	0	n.a.	1	0	0	30	30

(Pt. No. = Patient number; Age dg. = Age at diagnosis; gen/cen., gen/tel. = number of gene probe signals divided by the number of centromere or telomere signals; Histol. 2 or 3 = histological type ductal carcinoma grade G2 or G3, M = medullary carcinoma, L = lobular carcinoma, CS = cystosarcoma phylloides; IHC = immunohistochemistry; pT, pN and pM = number of tumors, nodes and presence of metastasis based on the TNM classification system; DFS = disease-free survival; OS = overall survival; mth. = months; n.a., data not available).

groups (P = 0.773; *BRCA1* versus non-*BRCA*, P = 1.000; *BRCA1* versus *BRCA2,* P = 1.000; *BRCA2* versus non-*BRCA,* P = 0.502). All three *ZNF217* amplified tumors were of the medullary histological type and were negative for ER and PR. Correlation with histological typing was statistically significant (P = 0.002). There were no significant correlations between *ZNF217* amplification and ER (P = 0.777), PR (P = 0.799), PCNA (P = 0.522) and ERBB2 expression (P = 0.404), or with TNM classification (P = 0.592, 1.000, and 1.000, respectively, for pT, pN and pM). Due to the low number of *ZNF217*-amplified tumors, it was not possible to assess any correlation with age, EGFR, disease-free and overall survival.

Discussion

We observed *CCND1* amplification in eight of the 40 tumors examined (20%). This detection rate is similar to those of a number of other studies, which have observed *CCND1* amplification in between 10-20% of samples. *CCND1* amplification was found in 18% of 88 cases of ductal breast cancer *in situ* [21], for example, and in 9.6% of 613 breast cancer cases studied by Elsheikh *et al.* [22]. Similarly, Letessier *et al.* [23] observed 11q13 (*CCND1*) amplification in 19.6% of 296 informative breast cancer cases.

CCND1 was amplified in three of the 15 (20%) *BRCA1* tumors, three of the nine (33%) *BRCA2* tumors, and two of 11 (12.5%) tumors with neither *BRCA1* nor *BRCA2* mutations. No significant differences were observed between the *BRCA1, BRCA2* and non-*BRCA* tumors. Our results for *BRCA1* tumors accord with those of Palacios *et al.* [17], who found *CCND1* amplification in 18% (two out of 11) of *BRCA1* tumors and 35% (20 of 56) of sporadic age-matched breast carcinomas. Palacios *et al.* found amplified *CCND1* in three out of five (60%) *BRCA2* tumors, the sample number was very low, however, and the results should be treated with caution [17]. Vaziri *et al.* [16], on the other hand, found no cases of amplification in 30 tumors from *BRCA1*-mutation carriers, though 19 of 74 (26%) tumors from non-familial breast cancer controls did show *CCND1* gene amplification (P = 0.001).

As regards *BRCA* status, there have been more studies focused on cyclin D1 expression than on *CCND1* gene amplification. Most of these studies have exhibited significantly lower cyclin-D1 expression levels in *BRCA1*- mutation carriers than in sporadic tumors [1, 9, 28]. The percentage of cyclin D1 expression ranges from 5 to 33% in *BRCA1* tumors, from 27 to 57% in *BRCA2* tumors and from 35 to 55% in non-*BRCA* tumors [7, 16, 24, 25, 26]. No difference in cyclin D1 expression was found in 157 hereditary breast cancers with *BRCA2* germline mutations and 314 control tumors negative for the *BRCA1* and *BRCA2* mutations [8].

Cyclin D1 is a member of a family of proteins that functions primarily in the cell division cycle, regulating the activity of cyclin-dependent protein kinases [27]. Cyclin D1 is essential for G1 phase progression [28] and is implicated in the pathogenesis of several human malignancies, including breast carcinoma [29, 30]. Cyclin D1 probably mediates many of the known hormonal effects on cell proliferation in breast epithelial cells [28]. In mice engineered to be cyclin D1 deficient, the normal mammary epithelial proliferative response to pregnancy is ablated, demonstrating in vivo the critical role that cyclin D1 plays in normal epithelial proliferation [31]. Transgenic mice overexpressing cyclin D1 in the mammary epithelium are characterized by abnormal mammary cell proliferation, including development of mammary adenocarcinomas [21, 32].

Cyclin D1 is induced by estrogen and its association with ER positivity has been clearly demonstrated in breast cancer [33, 34]. In crossed ER**α**-overexpression mice with cyclin D1 knockout mice, mammary gland morphogenesis was completely interrupted [33]. ER**α**-overexpressing cells are absolutely dependent on cyclin D1 for proliferation [35]. These results have implications for tumors with ER overexpression and are supported by the results of clinical studies demonstrating that cyclin D1 overexpression correlates with ER positivity in breast cancer [22].

According to *in vitro* studies, cyclin D1 expression is dependent on ER**α** signaling [34]. ER**α**-negative tumors and tumor cell lines that also show over-expression of cyclin D1 have also been found, suggesting that, in addition to ER**α** signaling, cyclin D1 expression is under the control of other signaling pathways. These pathways may even be over-expressed in the ER**α**-negative cells [34]. ER negativity is typical of tumors from *BRCA1* mutation carriers. Although the rate of cyclin D1 positive cases is lower in *BRCA1* tumors than in sporadic tumors, they still represent some 5 to 33% of all positive cases [7, 20, 24, 25, 36]. The aforementioned alternative pathways of cyclin D1 overexpression induction may be involved in these positive cases. Some cases may also be explained by *CCND1* amplification, which is a non-ER dependent mechanism.

Whereas all *BRCA2* and non-*BRCA* tumors with *CCND1* amplification in our study were ER positive, all three *CCND1* amplified *BRCA1* tumors were ER negative. Our results suggest, therefore, that if a tumor has both *CCND1* amplification and ER negativity, it may be associated with a *BRCA1* mutation. Thus, positive *CCND1* amplification and ER negativity may represent further features that may have implication for performing a *BRCA1* gene mutation analysis.

Similar to our results, two previous studies have noted a correlation between *CCND1* amplification and decreased survival of patients [1, 37].

Amplification at 20q13 occurs in a variety of tumor types and is associated with aggressive tumor behavior [38]. ZNF217, a Kruppel-like zinc finger transcription factor with oncogenic potential, is localized on this chromosomal region and is known to immortalize breast epithelial cells and to suppress cell death [39].

We found a weak *ZNF217* amplification in three of 38 (8%) tumors. All the tumors with *ZNF217* amplification were ER and PR negative and all of them were of the medullary histological type. Shimada *et al.* [40] detected *ZNF217* amplification

in six of 25 (24%) breast cancer cases and correlated this with higher clinical stages. In addition, Letessier *et al.* [23] observed 20q13^{Z} amplification (amplified at *ZNF217* only) in 9.9% of 233 informative cases and 20q13^{Co} (co-amplification of two or three 20q13 loci, i.e. *MYBL2, ZNF217* and *SKT6*) in 8.5% of 270 invasive breast cancer cases. Letessier *et al.* [23], therefore, observed *ZNF217* amplification in 18.4% of all cases examined. Amplification of 20q13^{Co} was correlated with a high grade of malignancy and axillary lymph node invasion and 20q13^{Z} amplification with ER and/or PR negativity [23].

Ginestier *et al.* [2] observed 36 of 466 (8%) tumors with 20q13 (*MYBL2, ZNF217* or *SKT6*) amplification, *ZNF217* being amplified in 31 cases (7%). In nine of the 36 samples, *ZNF217* was amplified without amplification of the two other loci. *ZNF217*-only amplified tumors were associated with an absence of lymph node metastasis and, paradoxically, with a poor prognosis, whereas tumors with amplification at two or three loci were associated with high histological grade, presence of lymph node metastasis and, paradoxically, with a good prognosis. A strong correlation existed between the two classes and the ER status of tumors, with more ER-negative tumors in the *ZNF217*-only amplified group as compared with amplification at two or three loci.

Two of the *ZNF217* amplified tumors were from *BRCA1* gene mutation carriers and one from a patient with no *BRCA1* or *BRCA2* mutation. There was no significant difference in *ZNF217* amplification between the groups. In breast tumors from *BRCA1* germline mutation carriers, high levels of *ERBB2* oncogene amplification do not occur, or occur only rarely. In contrast, *ERBB2* is highly amplified in approximately 15% of sporadic breast tumors [41]. There are several possible mechanisms to explain why *ERBB2* is never highly amplified in the background of a *BRCA1* germ-line mutation. One is that impaired BRCA1 may functionally suppress amplification. BRCA1 contains several functional domains that interact directly or indirectly with a variety of molecules [42], and is likely to serve as an important central component in multiple biological pathways [43], including processes involved in gene amplification. Some gene amplification processes require functional nonhomologous end-joining [reviewed in 9, 44]. Loss of this repair pathway in BRCA1 mutant tumors may limit gene amplification [reviewed in 9]. Our results do not support this hypothesis, however, as we found *CCND1* and *ZNF217* gene amplification in similar proportions of *BRCA1, BRCA2* and non-*BRCA* tumors. Other possible mechanisms that block *ERBB2* amplification in *BRCA1* tumors are more probable, therefore, including co-deletion of one allele of *ERBB2* and its nearby sequences during loss of heterozygosity at the BRCA1 locus, thus disabling gene amplification, which requires the presence of both alleles of the gene or amplification suppression through an indirect structural mechanism, such as abnormal chromatin conformation on 17q [9, 45–47].

In none of our samples, co-amplification of *CCND1* and *ZNF217* was observed. Our study sample was too small, however, and the number of loci analyzed too incomplete, to make any further conclusions. Letessier *et al.* [23] observed a frequency for single locus amplification of 1.6% for 20q13^{Z} and 20q13^{Co}; in all other cases, co-amplification was detected with one or more other loci (8p12, 8q24, 11q13 or 17q12). The 11q13 region was never observed to be amplified alone [23]. Our *CCND1* or *ZNF217* amplified tumors, therefore, may also be amplified at another locus.

In conclusion, our results show that *CCND1* amplification can be associated with a worse outcome of breast cancer. Concurrent *CCND1* amplification and estrogen and progesterone receptor negativity may be a predictor of germline *BRCA1* gene mutation. This observation should be confirmed in a larger study, however. The equal frequency of *CCND1* and *ZNF217* amplification in *BRCA1, BRCA2* and non-*BRCA* breast cancer suggests that loss of functional BRCA1 protein may not limit gene amplification.

Acknowledgements: This work was supported by the Internal Grant Agency of the Ministry of Health Care of the Czech Republic, project No. NR/9092-3.

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