Chromosome 17 centromere amplification and chromosomal instability (CIN) in breast cancer: Pathogenic and therapeutic implications

Minireview

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Chromosomal instability (CIN) is present in variable degrees in a significant percentage (up to 90%) of cancers and often portends adverse outcomes. However, it has not been incorporated in clinical practice as a prognostic marker due to the lack of standardization and proof of clinical utility of assays to measure it, as well as uncertainties regarding optimal cut-offs. Amplification of the centromeric region of chromosome 17 as measured by In Situ Hybridization (ISH) of the CEP17 probe is used clinically as part of the ISH assay for HER2 status determination in breast cancer in cases with intermediate (2+) result of HER2 protein expression by immunohistochemistry. CEP17 amplification concerns the centromeric area and rarely extends beyond it to involve polysomy of the whole chromosome. The association of CEP17 amplification with generalized CIN remains uncertain. Such association, if confirmed, could be an opportunity for a practical and clinically validated test of CIN in breast cancer. This paper explores the association of CIN with centromere 17 amplification and with centromere function in general, as well as the pathophysiology of centromeres/kinetochore function during mitosis that underlies their relationship with CIN in cancer and in breast cancer in particular.

Key words: chromosomal instability, aneuploidy, breast cancer, centrosome 17, prognosis

Chromosomal instability (CIN) is defined as a gain or a loss of chromosomal material in one or more chromosomes. Gain or loss of whole chromosomes is referred to as aneuploidy or numerical CIN (polysomy or monosomy). Amplifications or deletions containing only parts of chromosomes are referred to as structural CIN [1]. Most commonly, numeric aneuploidy is associated with structural aneuploidy through a production of micronuclei that contain lagging chromosomes produced through mitotic defects. These micronuclei may subsequently break down and release their fragmented DNA content that can become re-incorporated in chromosomes of the main nucleus [2]. CIN is common in cancers and its incidence in breast cancer is high. In a study, for example using centromere 2 and 15 probes, 36.9% of cases (82 of 222 patients) had deviations from the modal number in these centromeres in 30% or more of cells [3]. In another study, 62.3% of 313 breast cancer samples showed gains in either chromosome 1 or chromosome 8, or both [4].

The common amplification of ERBB2 locus in breast cancers, that occurs in about 15% of these cancers and defines a clinical sub-type, is an example of a structural aneuploidy. In these cancers a genomic area surrounding the ERBB2 locus, termed ERBB2 amplicon, is amplified without amplification of loci in chromosome 17 beyond the amplicon [5, 6]. The size of the ERBB2 amplicon is variable. It has been observed that, as expected, genes localized closer to the ERBB2 locus are most commonly co-amplified compared with more distantly located genes at 17q12-21 loci [7, 8]. Similarly, cases that show amplifications of the centromeric area of chromosome 17, as identified with the CEP17 probe (Centromere Enumeration Probe 17), concern almost always amplification of this area only, and are very rarely associated with polysomy 17 [9–11]. According to the ASCO-CAP (American Society of Clinical Oncology and College of American Pathologists) definition of ERBB2 amplification for clinical use, if there exists a more widespread amplifica-

tion or co-amplification that includes the centromeric area of chromosome 17 (CEP17) and thus producing an ERBB2 to CEP17 ratio in dual probe ISH evaluation of less than 2, ERBB2 is considered non-amplified except if its absolute copy number exceeds 6 [12]. This definition attempts to capture the fact that cancers with widespread amplifications may derive reduced or no benefit from HER2-targeting treatments, despite increased ERBB2 copy numbers. The possible different pathogenic implications of more extensive amplifications contributing to this resistance remain ill-defined. Reduced benefit of HER2-targeting treatments in patients that present with concomitant increase of copy numbers of CEP17 region may imply different pathogenesis that makes these cancers less dependent on function of the amplified HER2 protein and thus less sensitive to its inhibition. Alternatively, increase of copy numbers of CEP17 region may denote an underlying higher degree of generalized CIN that results in HER2-targeting treatments resistance. This is despite the fact that even ERBB2 amplified, as clinically defined, breast cancers contain an increased number of concomitant chromosomal gains compared to non-amplified counterparts [13]. This paper will discuss the role of centromere 17 alterations in breast cancer both regarding pathogenesis and therapeutic implications. Associations of centromere 17 alterations with CIN will also be discussed.

Centromere 17 amplification in breast cancer: Associations and prognostic implications

Amplification of the centromeric region of chromosome 17 is measured in clinical samples of breast cancer by ISH using the probe D17Z1 that hybridizes with α satellite DNA repeats present in tandem arrays across human chromosome 17 centromere. D17Z1 arrays are the site of kinetochore assembly of human chromosome 17 during mitosis in most cases, although a back-up array assembles the kinetochore in a minority of cases [14]. Active centromeric regions that assemble kinetochores during mitosis play a critical role in correct spindle construction and equal chromosome distribution in daughter cells [15]. Thus, cancers with CEP17 numeric abnormalities may have a parallel general-

ized defect in the mitotic machinery associated with CIN (see also next section).

The CEP17 probe is used in the evaluation of patients with HER2 intermediate scores in immunohistochemistry (IHC 2+) who require ISH evaluation in order to resolve HER2 status for therapeutic decisions in the clinic [16, 17]. According to established guidelines, breast cancers with a score of 2+ in IHC undergo dual probe ISH (probes for HER2 and CEP17) and are considered positive if either the ratio of HER2 to CEP17 is above 2 or if there is an average of more than 6 signals of HER2 per tumor cell nuclei independently of the number of CEP17 signals [12]. Although HER2 positive tumors as defined by these criteria form a well-characterized subset of breast cancers with treatment implications (addition of HER2-directed therapies in treatment regimens), they have significant underlying genetic heterogeneity [18]. In addition, the repercussions of gains of CEP17 (with or without HER2 abnormalities) in breast cancer are less clear.

Several studies have attempted to characterize cancers with the amplification of CEP17 and to derive prognostic information from this molecular abnormality. Amplification of chromosome 17 centromere is present in about 10% to 40% of cases and appears to be associated with intermediate prognosis between HER2 negative and HER2 positive tumors [19–21]. Its prevalence is higher in breast cancers with amplification of the ERBB2 gene at locus 17q12 encoding for HER2 than in ERBB2 non-amplified tumors in some studies but shows no correlation with amplification of the ERBB2 locus in several others. A selection of studies of CEP17 amplification in breast cancer is discussed below and presented in Table 1.

Compared with HER2-negative breast cancers, breast cancers with CEP17 gain (in the study of Ji et al. defined as higher than 3.76 CEP17 copies per nucleus and not fulfilling the ISH criteria for HER2 positivity [20]) were significantly more often grade 3 (12.5% versus 8%, p=0.04), were more often 2+ or 3+ by HER2 IHC (80.4% and 8.7% versus 57.2% and 2.8% respectively, p=0.000) and had more often a Ki67 positivity status above 20% (47.3% versus 36.8%, p=0.03) [20]. In addition, Disease-Free Survival (DFS) of patients with CEP17 gains was intermediate between patients with

Table 1. Examples of studies of CEP17 amplification in breast cancer. DFS: Disease Free Survival, LVI: Lymphovascular Infiltration, OS: Overall Survival, PFS: Progression Free Survival, NR: Not Reported. Cut-off refers to average copy numbers per nucleus.

Reference	Number of patients	Cut-off	Prevalence (%)	Associations	Prognosis
Ji et al. [20]	770	3.76	23.9	Ki67, grade	PFS intermediate
Lee et al. [21]	945	3.0	19.7	Ki67, grade, LVI	DFS worse in ER+/HER2-
Petroni et al. [22]	647	3.7	27.3	Ki67	NR
Jiang et al. [23]	109	2.6	33.9	ERBB2 amplification, grade	NR
Liu et al. [24]	348	3.0	13.8	ER	NR
Foutzilas et al. [25]	1031	3	39.9	ERBB2, TOP2A	No OS difference
Gogas et al. [26]	229	3.22	10.9	ERBB2 amplification	NR
Salido et al. [27]	175	3.0	12.5	Nodal status	No association with relapse

HER2 negative tumors who had the best DFS and those with HER2 positive tumors who had the worse DFS [20]. Another group found that CEP17 gain (defined as a copy number of ≥3.0 in this study) was present in 19.7% of breast cancers in their series of 945 patients [21]. CEP17 gain was associated with a worse DFS in the HER2 negative group but not in the HER2 positive group. No differences in DFS in either groups were noticed when a different CEP17 gain cut-off of 2.6 was used [21]. The DFS difference with the CEP17 cut-off of 3 was due to differences in the ER positive subgroup, while triple negative patients had no different DFS if they had concomitant CEP17 gain compared with no CEP17 gain. However, the number of ER and HER2 negative patients included in the series was small.

Another series showed CEP17 gain (defined as a copy number of ≥3.7) in 27.3% (177 of 647 patients) of breast cancer patients [22]. This study found an association of cases with CEP17 gain with higher proliferation index as measured with Ki67 compared with cases without CEP17 gain. No associations with age, tumor size, nodal status or grade were discerned. Similarly, no statistically significant associations of ER status or ERBB2 amplification between cases with CEP17 gain compared with those without CEP17 gain were observed [22].

CEP17 amplification was defined as equal or more than 2.6 signals per cell in a study of 109 breast cancer patients and was associated more often with ERBB2 amplification (60% versus 25% in cases with no CEP17 amplification) and high grade nuclear atypia than CEP17 non-amplified cases [23]. An association of CEP17 amplification with 3+ HER2 staining in immunohistochemistry was observed in this group [23].

Another series of 348 breast cancer patients from China showed a prevalence of CEP17 amplification (>3 signals/nucleus) of 13.8% [24]. There was a poor correlation of CEP17 amplification with ERBB2 amplification. Among patients with 6 or more ERBB2 signals per nucleus, 33.7% had also CEP17 amplification (>3 signals/nucleus), while these percentages were 87.5% and 4% for the groups with 4 to 6 and less than 4 ERBB2 signals/nucleus, respectively [24]. Cases with CEP17 amplifications were more commonly ER positive (66.2%) than HER2 positive patients (defined by an ERBB2/CEP17 ratio >2.2) who were ER positive in 42.2% of cases.

In a report of over a thousand breast cancer patients across sub-types that had participated in two adjuvant anthracycline trials of the Hellenic Cooperative Oncology Group (HeCOG), 39.9% of patients presented gain in CEP17, defined as more than 3 signals per nucleus in at least 30% of counted nuclei [25]. CEP17 gains had a higher prevalence in cases with increased numbers of ERBB2 signals and cases with increased numbers of TOP2A signals. Interestingly, cases with TOP2A deletions had also an increased occurrence of CEP17 gains compared with TOP2A non-amplified cases. Investigators reported also on CEP17 gain prevalence according to biological subtype. Luminal A cancers (ER-positive and/or PR-positive, HER2-negative and Ki-67 <14%) had

CEP17 gain in 38.4% of cases. Similarly, luminal B cancers (ER-positive and/or PR-positive, HER2-negative and Ki-67 >14%) had CEP17 gain in 39.4% of cases. In HER2-amplified tumors gains of CEP17 were observed in 49.3% ER-positive and 53.7% of ER-negative patients. Triple negative sub-type had a CEP17 gain in 26.2% of cases [25]. No differences in survival were observed according to CEP17 status.

A series of breast cancer patients that had been treated in HER2-positive trials of HeCOG had been investigated for copy number alterations of c-MYC, c-MET, ERBB2, TOP2 and the centromeres of the corresponding chromosomes, CEP8, CEP7 and CEP17 [26]. Some of these patients had actually been determined to be HER2 negative by central review. TOP2A amplifications and c-MYC amplifications were observed more commonly in centrally HER2-positive tumors (46.4% and 21.8% of cases) than in centrally HER2-negative cases (6.8% and 11.1% of cases respectively). In addition, HER2 amplified tumors by central review had more commonly amplifications of CEP17 (13.8% versus 6.8%) than tumors that were centrally HER2 non-amplified. In contrast no differences in amplification frequencies of CEP7 or CEP8 were observed. CEP8 was amplified in about one fourth of both HER2 positive and negative cases [26]. Thus, it appears that a significant percentage of breast cancers bear abnormalities in centromeric chromosomal areas independently of their ERBB2 amplification status.

In a study of CEP17 "polysomy", defined as 3 or more copies per nucleus, in 175 breast cancer patients, the incidence of CEP17 gain was 12.5%. No association of CEP17 gain with ERBB2 amplification has been observed [27]. CEP17 amplification was present in 15% ERBB2 non-amplified cases and in 10% of ERBB2 amplified cases. No associations were also observed between CEP17 gain and either tumor grade or ER status [27].

Data discussed here show a high prevalence of CEP17 amplification in breast cancer and argue for a possible prognostic role of CEP17 amplification in at least sub-sets of breast cancer. If the underlying pathophysiology of CEP17 abnormalities is related to a more generalized CIN in some cases, as it will be discussed next, it could be explored for obtaining therapeutic gains. However, the fact that studies that examined other centromeric amplifications found them to be present in breast cancer commonly in the absence of CEP17 amplification may suggest that use of a combination of centromeric and arguably non-centromeric probes may be preferable for this purpose. In this line of argument clinical cases with both ERBB2 and CEP17 amplifications may have a higher CIN level than cases with only one of them or none of the two probes amplified.

Interestingly from the limited and somewhat heterogeneous data available, no clear association of ER status of the tumor with CEP17 gains is observed. Studies have shown either no statistically significant association or association of higher rates of CEP17 gains with ER positive cancers [24, 27] or conversely a higher rate of CEP17 gain in HER2 positive

cancers than in the luminal types [25]. These data suggest that either there is no strong association of CEP17 gains with breast cancer sub-types or the heterogeneity of the studies both regarding populations included and techniques used prevent identification of sub-types differences in this regard. In addition, CEP17 gain is observed in cancers across the spectrum of HER2 expression by IHC and thus it seems that it does not significantly influence such expression, despite the proximity of the two loci [28]. In the study of Downs-Kelly et al. 69% of patients with CEP17 gain were negative for HER2 (0 or 1+ by IHC), 27% of patients had an intermediate score of 2+ and only 3% of patients had an HER2 expression of 3+ [28]. Clearly further studies are required to confirm CEP17 role in breast cancer.

Centromeres integrity and CIN in cancer

The repetitive nature of centromeric DNA presents challenges in the maintenance of integrity and length of centromeres during their duplication before distribution to daughter cells [29]. Thus centromeric and pericentromeric regions are common sites of breaks and re-arrangements. Cancer-associated re-arranged chromosome fragments tend to be better preserved in subsequent cell generations if they are connected to a functional centromere, while they are lost in subsequent mitoses and cell divisions if they are acentric [30]. Given that centromere assembly and functionality depends on epigenetic factors, such as histone 3 variant CENP-A occupancy rather than the underlying centromere specific α satellite sequences, CENP-A overexpression protects against DNA damage induced for example by UV light [31]. In addition, DNA double breaks produce accumulation of centromeric proteins, such as CENP-A and its chaperone HJURP (Holliday Junction Recognition Protein) in broken sites [32, 33]. This accumulation leads to the recruitment of additional repair proteins, such as hMSH5 and NBS, possibly connecting centromere production with DNA repair. Centromere formation in sites of DNA double strand breaks may constitute a back-up mechanism for rescuing DNA sequences if repair fails. Consistent with these considerations, mislocalization of CENP-A promotes CIN [34]. As a result, CEP17 amplification observed in some breast cancers as well as amplifications of other centromeres present concomitantly in the same cases or instead in other cases may become a feed forward mechanism of CIN stabilization by contributing to preservation of rearranged DNA fragments. However, CEP17 amplification as observed in breast cancer in the clinics requires the presence of amplified underlying α satellite sequences of the centromere 17, which are detected by the used ISH probe, while even functional centromeres able to assemble kinetochores would not be detected by the probe if not associated with the respective α satellites. Thus, in clinical cases with centromere amplifications the underlying α satellite sequences are amplified. Whether these amplified α satellites are able and do in fact assemble functional

kinetochores and constitute the basis for functional centromeres remains to be clarified. Such functionality would favor survival of cancer cells with high CIN as it would protect DNA fragments from loss in daughter cells that could lead to decreased or loss of viability. For this reason, centromere amplifications may constitute an advantage for CIN-high cancers and be maintained through Darwinian selection in these cancers. However, the offered advantage may be centromere- and case-specific, depending on the sequences rescued (Figure 1A). Theoretically, if a new amplified centromere rescues a fragment bearing an important oncogene, it may provide significant advantage for the cancer cell survival and proliferation. In contrast, if the rescue operation of another amplified centromere happens to involve a fragment bearing a tumor suppressor or several tumor suppressors, it may have the opposite effect and be deleterious for the survival of the specific cancer cell. This might be the mechanism underlying the phenomenon of specific chromosomes centromere amplifications observed in some cancers [29].

Chromosome 17 centromere amplification: Relationships to CIN mechanisms

Evidence supports the association of amplifications in chromosome 17 centromere region and in the ERBB2 locus with generalized CIN, although given the higher CIN prevalence than the prevalence of CEP17 and ERBB2 gains in breast cancer, several cases without these gains may still have high levels of CIN. First, suggestive of CEP17 and ERBB2 association with CIN, there is an association of the amplification of the two regions as well as the locus of topoisomerase II gene, TOP2A, which is located telomeric to ERBB2 in arm 17q [35]. When CEP17 was amplified, ERBB2 was concomitantly amplified in 38.3% of cases, and TOP2A was amplified or deleted also in 38.3% of cases, while when CEP17 was not amplified, HER2 was amplified in only 15% of cases and TOP2A was amplified or deleted in 10% of cases [36]. In addition, in a study that used a panel of microsatellite markers for 26 chromosomal regions, ERBB2 amplified tumors had higher genomic instability (27% versus 19%) than non-amplified tumors [37]. In another study examining chromosome 17 alterations in breast cancer cell lines, HER2 positive cell lines had a higher frequency of chromosome 17 rearrangements [38]. ERBB2 amplifications have also been associated with the phenomenon of chromothripsis, which consists of catastrophic rearrangements in defined genetic areas, while the rest of the genome remains intact [39].

A second line of evidence for the association of centromere lesions with CIN comes from studies in model organisms, which have shown that re-replication errors of centromere regions that lead to replication of these regions more than once in a single cell cycle, induce CIN [40]. Additionally, transfection of lentiviral vectors expressing centromeric α satellite sequences in human mammary epithelial cells promoted segregation errors and copy number altera-
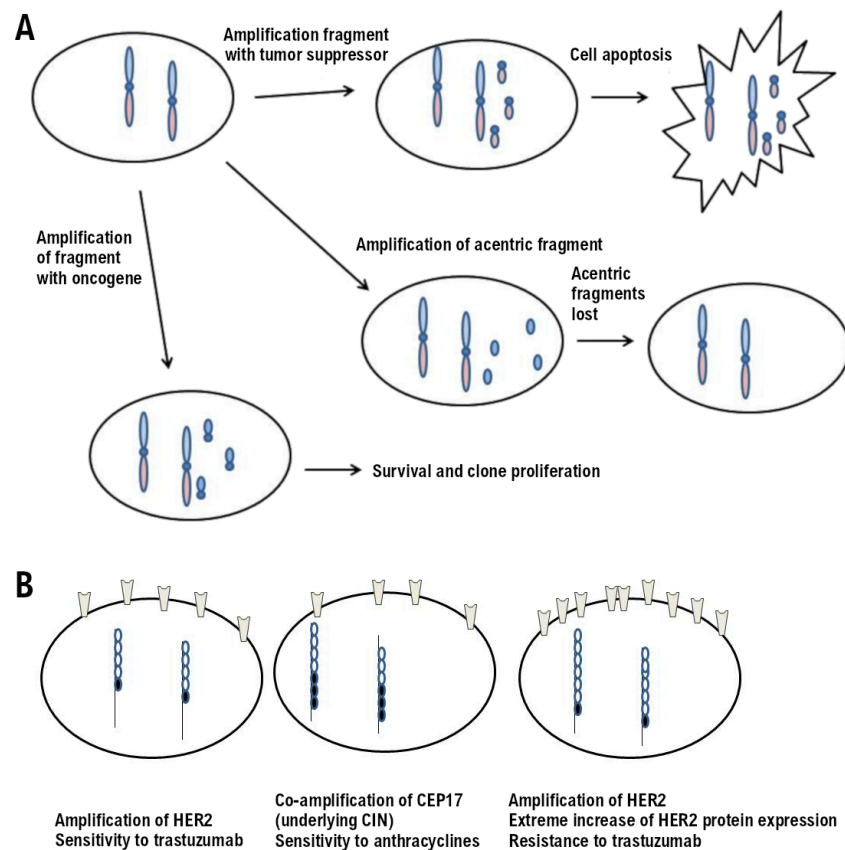
Figure 1. A) Schematic representation of possible effect of amplifications in the fate of a cancer cell. Amplification of genetic material containing a tumor suppressor (light red) would promote cell apoptosis (upper part). Amplification of material without associated functional centromere has a higher probability of loss of the amplified sequences if they are not re-incorporated in a chromosome (middle cell fate). Amplification of chromosomal parts containing oncogenes (light blue) would promote cell survival and proliferation and could be clonally selected. **B)** Effect of HER2 amplification in the sensitivity to treatments. Cancers with HER2 amplification without co-amplification of CEP17, implying lower CIN levels (left), are more sensitive to anti-HER2 based neo-adjuvant treatments. Co-amplification of CEP17, implying a higher level of CIN (middle), may underline increased sensitivity to anthracyclines. High levels of surface expression of the HER2 protein often produced by high levels of HER2 amplification (right) may lead to resistance to anti-HER2 treatments. Open ovals on chromosome 17 (represented by a vertical line) represent the HER2 locus and dark ovals represent the centromeric locus.

tions [41]. Overexpression of α satellites was associated with hypomethylation of these sequences. Moreover, loss of trimethylation at lysine 9 of histone 3 (H3K9me3) in pericentromeric chromatin was associated with a reduction of ability to assemble the kinetochore [42]. This would suggest a role of transcriptional silencing of the pericentromeric heterochromatin for the correct function of centromeres. Overexpression of demethylase JMJD2B that catalyzes H3K9 demethylation is associated with CIN in breast cancer and forced expression has the same effect in cell lines [42]. In contrast to the pericentromeric heterochromatic areas, centromeres bear post-translational modifications of potentially transcriptionally active chromatin, such as H3K4 dimethylation and lack transcription repressive H3K9 dimethylation [43]. Transcription of non-coding satellite centromeric areas play a role in assembling the functional centromere by producing long RNAs, which bind histone variant CENP-A carried by chaperone HJURP and contribute to the correct localization

of CENP-A in the centromere [44]. The length of these RNAs that may be over a kilobase long appears to be critical, as shorter transcripts accumulate under stress or upon differentiation conditions and lead to centromere dysfunction, possibly due to CENP-A mislocalization and to chromosome missegregation [45]. Overexpression of α satellite sequences has been reported in diverse murine and human cancer cell lines as well as cancer tissues compared to matched normal tissues [46].

A third, more circumferential line of evidence for an association of amplifications of ERBB2 and CEP17 loci with generalized CIN in breast cancers is provided by treatment efficacy data in the neo-adjuvant setting (Figure 1B). In a study from the Austrian Breast and Colorectal Cancer Study Group patients with an ERBB2 to CEP17 ratio higher than 6, implying lower numbers of CEP17 copies (as these are in the denominator) and thus possibly lower degree of generalized CIN, had a higher complete pathologic response rates (pCR)

to neo-adjuvant trastuzumab-based treatment than patients with ERBB2 to CEP17 ratios lower than 6 (pCR of 69% versus 30.4% in patients with ERBB2/CEP17 <6) [47]. Interestingly, ER positive tumors were the drivers of the ERBB2 to CEP17 ratio association with pCR while ER negative tumors had no statistically different pCR rates whether their ERBB2 to CEP17 was higher or lower than 6. In another retrospective study of patients receiving neo-adjuvant treatment for locally advanced HER2 positive breast cancers, patients with a higher ratio of ERBB2 to CEP17, as a continuous variable but not with a cut-off of 7, had a higher rate of pCR than patients with lower ERBB2 to CEP17 ratios and it was independently associated with pCR in a logistic regression model [48]. In this series only 373 of the 555 patients had received trastuzumab as part of their neo-adjuvant treatment. In another study of 140 patients that received anthracycline-based neo-adjuvant chemotherapy, patients with amplified CEP17, defined as a mean of more than 1.86 signals per nucleus, were associated with higher pCR to neo-adjuvant anthracyclines [49]. With the definition used in this study, 9.5% of the patients had CEP17 amplification. In addition, patients with amplification of topoisomerase IIα (TOP2A) showed a trend for higher pCR, while patients with ERBB2 amplification did not show different pCR compared to ERBB2 non-amplified cases. Given that anthracyclines have been proposed to be more effective in tumors with higher CIN [50, 51], these data may imply that CEP17 amplification is associated with CIN, and as a result, with anthracyclines sensitivity. Additional evidence from a pooled analysis of data from five adjuvant breast cancer clinical trials has indeed confirmed the predictive value of duplication of CEP17 and TOP2A aberrations, which may be surrogates for generalized CIN, for benefit from treatment with anthracyclines [52]. In contrast to the above data suggesting better clinical responses with higher ERBB2/CEP17 copy number ratios, a study that used a method of quantification of total HER2 protein content in breast cancers from the FinHer trial found that HER2 positive cancers by

ISH with the highest HER2 protein content showed resistance to trastuzumab [53]. This result suggests that caution is needed when evaluating associations in retrospective studies, especially in trials that have used combination treatments because a specific characteristic may promote response to one component of the combination while promoting resistance to another component. In treatments of HER2 positive breast cancers employing combinations of regimens containing anthracyclines and trastuzumab (or other anti-HER2 drugs), tumors with the higher ERBB2 amplification may be particularly sensitive to anthracyclines if significant CIN is present but resistant to trastuzumab if very high protein levels of HER2 receptor that are difficult to block are also present.

In summary, CEP17 amplification in the clinical context of breast cancer HER2 evaluation may give additional information regarding CIN and if present in the same tumors with ERBB2 amplification may suggest a more generalized pathophysiologic defect that leads to CIN in the specific breast cancer. HER2 evaluation alone may not inform on CIN globally as isolated amplifications in cancer promoting loci could be a chance event stabilized through selection of the amplification, which favors cancer cells survival and propagation given that it contains the ERBB2 oncogene. CEP17 amplified areas (and amplified centromeric areas from other chromosomes) could form functional kinetochores if associated with centromere specific histone CENP-A, which is often overexpressed in breast cancer as discussed in the next section.

Implications of pericentromeric chromosome 17 region abnormalities in breast cancer as derived from publicly available genomic data

Chromosomal centromeric loci are not fit for study by currently available genomic surveys because these studies rely on an assembly of fragments of variable sizes and sequences in order to call copy number alterations. The repetitive nature

Table 2. Pericentromeric genes in the long and short arms of chromosome 17. Centromere and pericentromeric heterochromatin of chromosome 17 span between about 22M and 27M. compl.: complementary chain.

Gene name	Description	Span	Gene ID number
p arm			
USP22	Ubiquitin Specific Peptidase 22	20999593-21043409 (compl.)	23326
TMEM11	Transmembrane protein 11	21197950-21214595	8834
MAP2K3	Mitogen activated protein kinase kinase 3	21284656-21315240	5606
KCNJ12	K⁺ voltage-gated channel subfamily J member 12	21376387-21419870	3768
q arm			
MIR4522	Micro-RNA 4522	27293910-27293996 (compl.)	100616277
WSB1	WD repeat and SOCS box-containing 1	27294021-27313631	26118
KSR1	Kinase Suppressor of Ras 1	27456470-27626438	8844
LGALS9	Galectin 9	27631148-27649560	3965
NOS2	Nitric oxide synthase 2	27756766-27800529 (compl.)	4843
LYRM9	LYR motif containing 9	27878314-27894746	201229
NLK	Nemo-like kinase	28042647-28205140	51701

of centromeric DNA precludes such an assembly in α satellite and pericentromeric regions. The status of centromere 17 (or any other centromere) copy numbers cannot thus be directly calculated in genomic studies. An indirect inference of the copy number status of centromeric areas could be attempted by studying the copy number status of genes located very closely to the centromeric and pericentromeric areas in both the q and the p arms of the chromosome. Copy number alterations of pericentromeric genes in both q and p arms in the same sample could suggest involvement of the centromere per se, in these samples, and thus serve as a surrogate marker of centromere sequences amplifications that are not directly testable with the current sequencing techniques. As an exercise to examine the feasibility of this approach and derive the amplification status of chromosome 17 centromeric loci, genes located closest to centromere in the 17q1.1 locus and 17p1.1 locus were identified (Table 2). Chromosome 17 is well suited to serve as the subject chromosome of this exercise in breast cancer given that its centromere has been examined in multiple studies with ISH techniques and is known to be amplified in 20% to 45% of breast cancer patients as detailed in a previous section. Some of the closest genes to centromeres in 17q1.1 listed after the pericentromeric gene desert in ensemble database include miR4522, WSB1, KSR1, LGALS9, NOS2, LYRM9 and NLK (Table 2). Similarly, genes located in the area neighboring the pericentromeric heterochromatin of the short arm of chromosome 17 include USP22, TMEM11, MAP2K3 and KCNJ12 (Table 2). However, and despite the comparative high frequency of centromere 17 amplifications, as determined by ISH, data from the METABRIC and TCGA Breast cancer studies examined in the cBioPortal platform [54–57] disclosed low frequencies of abnormalities in these pericentromeric genes and notably even lower frequencies of concomitant abnormalities in both genes located in p and q arms. In the METABRIC study that included over 1900 breast cancer patients, each of these genes was amplified in 2% to 4% of cases and all of them were concomitantly amplified in just six cases, of which only one was HER2-positive [57]. Similarly, the TCGA PanCancer Atlas Invasive Breast Cancer

study that included about a thousand patients disclosed CNAs in each of these genes in 2% to 5% of cases but only four cases had amplifications of all eleven genes in the short and long arm across the centromere 17 [56]. This suggests that amplifications of genetic material in chromosome 17 rarely extend across the centromere to include both short and long arms or the whole chromosome but constitute rather a quite localized phenomenon. These data agree with the aforementioned contention that CEP17 copy number gain in breast cancer is rarely due to chromosome 17 polysomy [10].

An additional approach for derivation of information on centromere status consists of studying genes known to be involved in correct centromere assembly, such as the specific centromere variant histone CENP-A, its chaperone HJURP, or protein CENP-B and CENP-C. Mutations or abnormal expression of each of these genes, as well as others involved in the same centromere assembly process, may be responsible for a sub-set of the observed cases with centromere copy number alterations and may contribute to stability of such centromere abnormalities by conferring functionality in the underlying repetitive DNA amplification. Given the importance of centromeres for prevention of aneuploidy, lesions in genes involved in correct centromere assembly would be expected to be associated with high CIN. Interestingly interrogation of the genomic TCGA PanCancer Atlas Invasive Breast Cancer study data on the cBioPortal platform disclosed that CENP-A lesions (mainly mRNA over-expression) observed in about 10.9% of cases segregate almost exclusively in the basal sub-type. Among the 112 patients with CENP-A over-expression, 92 (82.1%) belonged to the basal sub-type, nine patients (8.1%) had the luminal B sub-type, 11 patients (9.8%) were HER2 positive and no patients with CENP-A over-expression had a luminal A disease (Figure 2A). Conversely, among basal cancers in TCGA PanCancer study, 53.8% had CENP-A over-expression, while this percentage was 14.1%, 4.6% and 0 for HER2 positive, luminal B and luminal A cancers, respectively (Figure 2B). In the same study, the mean aneuploidy score for cases with a CENP-A over-expression was 15.67 (SD: 6.74) and with no CENP-A over-expression was 11.69 (SD: 7.83)
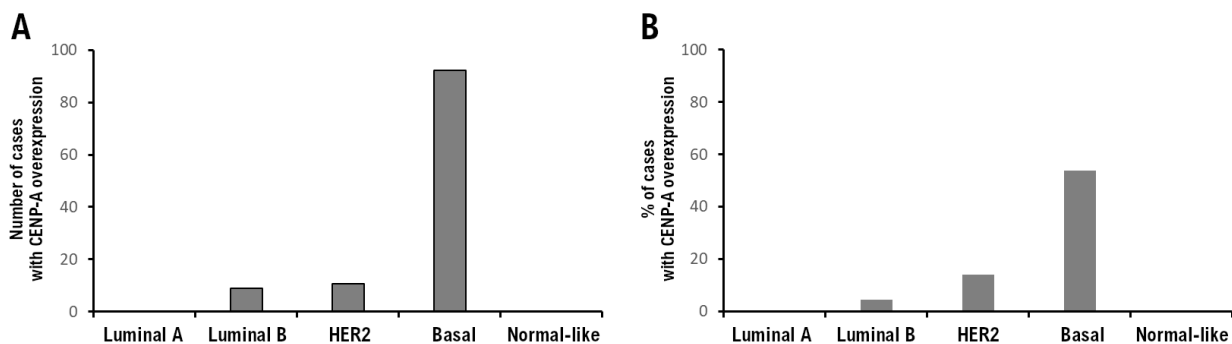

Figure 2. A) Absolute number and B) percentage of cases with breast cancer and CENP-A over-expression according to sub-type in the TCGA breast cancer PanCancer study.

(t-test p<0.001). CENP-A over-expression and mislocalization has been previously reported to be associated with CIN in cancer [34]. CENP-A chaperone HJURP mRNA overexpression displays also predominance in basal cancers. HJURP was over-expressed in 69 samples in TCGA PanCancer Atlas Invasive Breast Cancer among which 55 samples (79.7%) were basal, 10 (14.5%) were of the luminal B sub-type, 3 samples (4.3%) were HER2 positive, one (1.4%) normal-like and none of the luminal A sub-type. In contrast no differences in the aneuploidy score was observed between luminal B, HER2 positive and basal cancers, despite their significant differences in the frequency of CENP-A overexpression. Aneuploidy score depicts the number of each chromosome arm with significant copy number alteration [58]. Thus, the equal aneuploidy scores of HER2 positive and luminal B cancers with this of basal cancers despite their lower frequency of CENP-A and HJURP overexpression suggests that other abnormalities play a role in these sub-types or indeed that CENP-A and HJURP overexpression is an epiphenomenon downstream of other abnormalities leading to CIN. p53 suppresses expression of CENP-A and HJURP through CDE/CHR motifs in their promoters and this may contribute to its function as a CIN suppressor [59, 60]. Suppressive action of p53 is indirect in these promoters through upregulation of p21, which promotes repressive p107 and p130-containing DREAM complex recruitment [61]. As a result, mutations in p53 would lead to de-repression of the two genes. This de-repression is critical to prevent excessive CIN and cell death in cancers with p53 functional loss [59]. Interrogation of data from the TCGA PanCancer Atlas Invasive Breast Cancer at cBioPortal shows a high correlation of CENP-A mRNA upregulation with mutations in p53. Among the 112 samples with upregulated CENP-A mRNA only 11 had no concomitant p53 mutation. Moreover, consistent with the upregulation of CENP-A mRNA and the role of p53 mutations in this upregulation, p53 mutations are observed in 88% of basal breast cancers but only in 17% of luminal A cancers [62].

In contrast to the DREAM complex that binds CDE/CHR motifs and suppresses CENP-A promoter function, several other transcription regulators such as E2F1, MYBL2 (B-MYB) and FOXM may activate transcription through binding to the same motifs. All these three activating regulators have been found to be overexpressed in aneuploid breast cancers [63]. In addition, their overexpression in experimental models induces aneuploidy. The E2F family of transcription factors has members that activate and others that suppress their target promoters through binding to identical sequences and recruiting co-activators or co-repressor complexes [64]. Interestingly, a sub-set of patients from the TCGA PanCancer Atlas Invasive Breast Cancer at cBioPortal ranging from 5% to 28% have abnormalities (mostly mRNA upregulation) in one or more members of the E2F1 family including the inhibitory members. These abnormalities are positively correlated with both CENP-A and HJURP mRNA upregulation and p53 mutations (Benjamini-Hochberg's q<0.001). Thus, these data

suggest an effect of these transcription factors dysregulation in overexpression of centromeric histone CENP-A and its chaperone HJURP.

It is noteworthy that despite the robust association of these transcriptional regulators with centromeric protein abnormalities and the basal phenotype, CEP17 amplifications do not display any strong prevalence association with triple negative cancers [25]. In contrast, studies that measured instability in breast cancer samples have confirmed its association with basal or HER2-positive phenotypes [4, 65]. Thus, CEP17 amplification measurement alone may not be optimally depicting the CIN status of a tumor and a more global measurement is required.

Besides CENP-A and HJURP, several other proteins (MAD1L1, MAD2L1, MAD2L2, BUB1, BUB1B, BUB3, CDC20 and TTK) that associate with the kinetochore during mitosis and participate in mitotic checkpoint are overexpressed both at the mRNA and protein level in breast cancer cell lines with genomic instability and patient samples checked [66]. An association of increased expression of BUB1B protein with grade 3 cancers was also observed in this study. MAD2L1 overexpression, in addition to CENP-A overexpression, was critical in CIN development in cells with pRB loss which upregulates E2F1 [67, 68]. In TCGA PanCancer Atlas Invasive Breast Cancer samples these genes show abnormalities (mostly increased expression) in 5% to 12% of cases each. Basal cancers are over-represented in cases with overexpression of these mitotic regulators (data not shown).

Conclusions and perspectives

CIN, either numeric or structural and most often both, is very commonly present in cancer cells and the great majority of cancers display some degree of CIN. Its measurement in clinical samples has proven to be difficult to realize in a manner that will be clinically exploitable for diagnostic, prognostic and therapeutic purposes. CIN is a continuum of states and scales to assess and its quantification will need to be constructed and then validated before entered in standard clinical practice. Such measurements could be direct, consisting of evaluation of chromosome arm with copy number abnormalities akin to the aneuploidy score that currently is used only as a research tool [58] or indirect, consisting of measurement of genes associated with CIN, such as the CIN4 score proposed by Szász et al. [69]. The four genes included in this signature, AURKA, FOXM, TOP2A and TPX2 code all for important proteins in the mitotic spindle function and regulation.

Data discussed in this paper argue for the association of centromeric abnormalities and CIN and put forward the idea of centromere 17 amplification, possibly examined in association with other amplifications such as those of chromosome 2 and 15 [70], as a measure for CIN in breast cancer. CIN measurement could assist in breast cancer prognostication and therapy prediction, supplementing other methods.

The advantage of a well-defined quantitative measurement together with the fact that ISH-based methods are already used in the clinic could be an asset in such use, although clinical validation for the new use would still be required. Selection of additional centromeric probes besides CEP17 and optimal cut-offs for prognostication would be critical, especially given that the prognostic implications of CIN may not be linear [70, 71]. In addition, the implications of CIN might not be the same in different sub-types of breast cancer. Progress in answer these questions holds the potential of significant advancements in breast cancer prognosis and therapy.

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