Targeting the Spindle Assembly Checkpoint for Breast Cancer Treatment

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Targeting the Spindle Assembly Checkpoint for Breast Cancer Treatment

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Abstract

Breast cancer is the most common malignancy in women worldwide and the second leading cause of cancer death after lung cancer. Like in other malignancies, aneuploidy is a common feature of breast cancer and influences its behavior. Aneuploidy has been linked to inappropriate activity of the spindle assembly checkpoint (SAC), a surveillance mechanism that, in normal cells, prevents anaphase onset until correct alignment of all chromosomes at the metaphase plate is achieved. Interestingly, the widely used anti-microtubule drugs, vinca alkaloids and taxanes, kill cancer cells through chronic arrest in mitosis as a consequence of chronic SAC activation. Deregulated SAC has been reported in breast cancer in many reports and presents an attractive therapeutic strategy. We present here a review of the current knowledge on the SAC defects and the underlying molecular mechanisms in breast cancer, and discuss the potential of SAC components as targets for breast cancer therapies.
1. INTRODUCTION

Breast cancer is the most common malignancy in women worldwide [1]. Despite advances in detection and therapies, breast cancer remains the second leading cause of cancer death after lung cancer, stressing the urgent need to discover new therapeutic targets. Like other malignancies, it results from stepwise genetic and/or epigenetic alterations influencing crucial cellular pathways involved in growth and development of normal cells. A common genetic feature of cancer cells is aneuploidy that often occurs because of chromosome mis-segregation during cell division [2].

Accurate segregation of chromosomes at the metaphase-anaphase transition relies on their correct bipolar attachment to microtubules of the mitotic spindle and subsequent alignment at the metaphase plate. Unattached or improperly attached chromosomes activate a quality control mechanism called the spindle assembly checkpoint (SAC) which inhibits the onset of anaphase until attachment errors are corrected thereby ensuring error-free chromosome segregation. Damage to the SAC leads to aneuploidy during tumorigenesis as a result of partial loss of checkpoint function mainly due to changes in SAC protein levels in human cancers [3,4]. Interestingly, up to 80% of breast cancers are aneuploid and SAC deficiency was correlated with this aneuploidy [5].

Over the last decade, several reports have addressed the SAC activity and the underlying molecular alterations in breast cancer, providing compelling evidence of its prognostic value in breast tumors. Importantly, and as targeting SAC provides several approaches for selective killing of cancer cells, increasing reports have proposed appealing therapeutic strategies for breast cancer treatment. In this review, we summarize the basic mechanisms of SAC and highlight the findings that reported its deregulation in breast cancer, as well as those that exploited it as a potential target for breast cancer therapy.

2. BREAST CANCER

Breast cancer is, by far, the most frequent and the leading cause of cancer death in women and represents the second most common cancer worldwide, with an estimated incidence of 25% (1.67 million of new cases diagnosed), in 2012 [6]. On the basis of clinically relevant expression levels of the estrogen receptor (ER), progesterone receptor (PR), and the human epidermal receptor 2 (HER2), breast cancers can be subdivided into three major subtypes: ER- and/or PR-positive breast cancers; HER2-amplified breast cancers; and triple-negative breast cancers (TNBC) that lack ER and PR expression, and HER2 overexpression [7,8]. Histopathological and gene expression profiling defines many other subtypes of breast cancers, reflecting the heterogeneity and the
complexity of the disease, and conditioning the therapeutic approaches and outcome prediction [8,9]. Depending on the subtype, breast cancer is frequently treated with hormone therapy, chemotherapy, and targeted therapy, which inhibit cancer progression through a variety of different mechanisms [10]. For instance, hormone receptor-positive breast cancers are treated by estrogen-focused therapy, using tamoxifen (ER modulator) and fulvestrant (selective regulator of ER) [11], while HER2-positive breast cancer can be treated with anti-HER2 trastuzumab (Herceptin) antibody therapy [12,13]. TNBC are not amenable to hormone therapies or anti-HER2 agents, and are frequently treated with a combination of surgery, radiotherapy and standard chemotherapy [10]. Mutated or amplified genes are the main causes of either inherited or sporadic breast cancers. Amongst the genes affected are the tumor suppressors BRCA1, BRCA2, and p53, whose mutations compromise DNA repair and DNA damage-induced apoptosis thereby resulting in further genetic abnormalities and malignancy; and the protein phosphatase PTEN whose mutations affect the PI3K/Akt pathways resulting in increased cell proliferation and reduced cell death [14]. Amplification or overexpression of HER2 constitutes another important genetic cause of breast cancers as it induces changes in downstream pathways such as Raf/MEK/ERK and PI3K/Akt/mTOR thus affecting cell proliferation and survival [14].

The identification of these driving genetic alterations and signaling pathways was crucial for the development of molecular targeted agents such as those mentioned above. Nevertheless, and owing to the dynamic, rapid, and adaptive nature of cancer progression, resistance to all existing therapies provides continuous and challenging intellectual exercises and opportunities for searching additional targets, to offer more effective therapeutic options. In this regard, targeting components of the SAC spindle assembly checkpoint has emerged as an attractive therapeutic avenue of exploration of new cancer therapies [15], namely against breast cancers.

3. AN OVERVIEW OF THE SPINDLE ASSEMBLY CHECKPOINT

The great challenge of a dividing cell is to ensure that the progeny receives the right number of chromosomes, in order to maintain genomic integrity. In eukaryotic cells, accurate chromosome segregation at metaphase-anaphase transition is under the control of the SAC spindle assembly checkpoint (SAC), a safeguard mechanism that monitors the attachment of chromosomes, through their kinetochores, to microtubules of the mitotic spindle [4,16]. This mechanism prevents precocious anaphase onset whenever unattached or mis-attached kinetochores are present. Indeed, unattached or improperly attached kinetochores act as platform to generate the mitotic checkpoint complex (MCC), composed of the SAC proteins BubR1, Bub3, and Mad2 associated with the Cdc20, an activator of the E3 ubiquitin ligase anaphase promoting complex or cyclosome (APC/C) [17,18]. As a
consequence, APC/C<sup>Cdc20</sup> is inhibited which, in turn, prevents the ubiquitination and proteasome degradation of the mitotic subtrahs securin and cyclin B, leading to mitotic arrest (Fig. (1)) [4,19]. Once the last chromosome establishes bipolar attachment to the mitotic spindle and becomes aligned at the metaphase plate, the SAC is silenced through disassembly of MCC, leading to APC/C<sup>Cdc20</sup> activation and subsequent targeting of securin and cyclin B for degradation by the 26S proteasome [20]. As a result, separase, now dissociated from its inhibitor securin, cleaves the cohesion complex that holds sister chromatids together, thus allowing anaphase onset. In a synchronized way, cyclin B degradation leads to cyclin-dependent kinase 1 (CDK1) inactivation and the cell can exit mitosis (Fig. (1)) [20]. Additionally to MCC proteins, other checkpoint components have a relevant role in SAC signaling and effective response, namely the kinases Bub1, Mps1 and Aurora B, and the Mad1 protein [16]. This latter protein is involved in the diffusion and amplification of the SAC inhibitory signal that emanates from unattached kinetochores and diffuses throughout the cytoplasm in order to maximize APC/C<sup>Cdc20</sup> inhibition [21].

This signal amplification relies on the existence of two Mad2 conformations, closed (c-Mad2) and open (o-Mad2). According to the current model, Mad1 and c-Mad2 form a stable complex at unattached kinetochores, serving as a catalytic scaffold to recruit and convert cytosolic o-Mad2 into c-Mad2, which, in turn, is able to bind Cdc20 and prevents APC/C activation [21]. Moreover, c-Mad2 was reported to induce Cdc20 conformational change, allowing its binding with the BubR1 N-terminal, previously bound to Bub3, and thereby inhibiting the APC/C<sup>Cdc20</sup> [22]. Upon the alignment of all chromosomes at the metaphase plate, the existing MCC is extinguished and the assembly of new MCC is prevented, a process known as SAC silencing [23,24]. As a consequence, free Cdc20 activates the APC/C<sup>Cdc20</sup> which in turn targets securin and cyclin B for degradation, allowing anaphase initiation and mitotic exit, respectively.
Comentário [H1]: Figure 1 to be considered.
**Fig. (1). A molecular view of SAC signaling.** (A). Unattached (a) or incorrectly attached (b) kinetochores activate the SAC (SAC ON). At kinetochore, Mad1/c-Mad2 complex recruits and coverts cytosolic o-Mad2 into c-Mad2 which associates with Bub3, BubR1, and Cdc20 to form the MCC that inhibits the APC/C, preventing degradation of securin and cyclin B thus leading to mitotic arrest. (B). Upon attachment of all kinetochores, the SAC is turned off (SAC OFF). MCC disassembles and is no more generated, which leads to activation of APC/C and mitotic exit. Then, securin is degraded and separase can degrade cohesins between sister-chromatids leading to anaphase onset, while cyclin B degradation leads to CDK1 inactivation and mitotic exit.

Genome integrity relies on the accuracy of chromosome segregation at every cell division. Indeed, chromosome mis-segregation is thought to be the major driving force of chromosomal instability (CIN) that leads to aneuploidy (chromosome gains or losses), a common feature of many cancers [25]. CIN is a major contributor of tumor development, progression and aggressiveness, and is generally associated with poor prognosis. Besides the presence of extra centrosomes, sister-chromatid cohesion defects, and erroneous kinetochore-microtubule attachments, impaired SAC is also believed to be a common cause of chromosome mis-segregation (Fig. (2)) [26]. Accordingly, many tumors with high frequency of aneuploidy were reported to harbor deregulated SAC activity [4]. SAC components are now regarded as promising targets for anticancer drug development, and some drugs are at different stages of clinical trials (see below).
Figure 2 to be considered.
Fig. (2). Different cell fates according to SAC activity status. Cells with a functional SAC (left) detect unattached or mis-attached kinetochores and an arrest in mitosis is sustained until correct attachment is achieved, resulting in accurate chromosome distribution between daughter cells (each with 2N DNA content). In cells with a weakened SAC (middle), a sustained arrest in mitosis could not be ensured in the persistence of errors in kinetochore attachment and, mis-segregation occurs resulting in aneuploid daughter cells (2N-1; 2N+1). Cells with complete loss of SAC activity (right) exit mitosis prematurely, and die as a result of massive aneuploidization.

4. SAC GENE EXPRESSION AND ITS CLINICOPATHOLOGICAL SIGNIFICANCE IN BREAST CANCER

Aneuploidy is a common feature of breast cancer [27], implying that breast cancer cells display high rate of chromosome gain and loss. As mentioned above, impairment of SAC activity accelerates the rate of chromosome mis-segregation and thus constitutes a common mechanism that fuels aneuploidy. A large number of studies reported SAC deregulation in breast cancer, shedding some light on the mechanism of CIN in this disease. SAC gene expression signatures were uncovered and proposed as possible diagnostic or prognostic markers and, hopefully, as potential novel targets for breast cancer treatment (Table 1) [28]. Of note, and contrary to what was initially expected, SAC gene mutations at the DNA sequence level are rather rare [4]. Instead, SAC genes are
frequently altered at the transcriptional level. Next, we will restrict our analysis to those proteins that form the core components of the SAC pathways. Information on others components that cross the SAC pathway could be found in (Table 1).

4.1. Bub1

Bub1 (budding uninhibited by benzimidazoles 1) is a serine/threonine protein kinase that binds to kinetochores, and is involved in SAC signaling in response to a lack of tension on kinetochores [14]. Bub1 directly phosphorylates Cdc20 which decreases the activity of APC/C<sup>Cdc20</sup> thus contributing to SAC-mediated mitotic delay [29]. Bub1 was found to be overexpressed in breast cancer [30,31], and such expression was reported to associate with cancer proliferation and progression, and with a poor prognosis [32,33]. Nuclear Bub1 immunohistochemical status was suggested as an independent prognostic factor for both disease-free and breast cancer-specific survival of breast cancer patients [32,34,35].

4.2. BubR1

BubR1 (Bub-related 1) is a serine/threonine protein kinase that binds to kinetochores, and is involved in SAC signaling in response to a lack of tension on kinetochores. BubR1 is a component of the MCC (see previous section) and was reported to be overexpressed in breast cancer [30,31]. BubR1 overexpression was correlated with poor survival in early stage breast cancer patients [32,33,36,37], with the proliferation marker Ki-67, and with tumor grades II and III [30,37]. Interestingly, BubR1 was found to interact, in a two hybrid screen, with the breast cancer-specific gene (BCSG1) that is highly associated with the development and progression of breast cancer [38]. BCSG1 expression reduces that of BubR1, suggesting that it accelerates cancer progression by compromising SAC activity. Also, BRCA-2 promotes BubR1 acetylation required for SAC activity in mice [38,39]. Hence, decreased BRCA-2 induces weekend SAC activity, providing an elegant demonstration for how mutation in BRCA-2 leads to CIN without apparent mutations in SAC components [39]. Since BCSG1 and BRCA-2 are involved in early-onset cancer, BubR1 could be an attractive biomarker for early breast cancer detection.

4.3. Bub3

Bub3 is a crucial component of the MCC, is required for kinetochore localization of Bub1 and BubR1, and plays an important role in correct attachment of chromosomes to the mitotic spindle [17,18]. Although Bub3 overexpression was found in breast cancer cell lines and tissue samples [28,30], its clinicopathological
significance is still missing most likely because of the lack of high quality validated anti-Bub3 antibodies for immunohistochemistry.

4.4. Mad1

Mad1 (mitotic arrest deficient 1) serves as a functional receptor for Mad2 at unattached kinetochores. Kinetochore Mad1-Mad2 heterodimers promote the conformational activation of the cytosolic pool of Mad2, which induces signal amplification required to sustain mitotic arrest [21]. Mad1 overexpression was found in breast cancer cell lines and tissue samples [28,30]. Overexpression of Mad1 was correlated with HER-2 status, cancer subtypes, p53 status, and age [40]. Interestingly, high Mad1 expression predicted adverse prognosis and worst outcome for breast cancer patients treated with paclitaxel [40].

4.5. Mad2

Mad2 (mitotic arrest deficient 2) is a key component of the MCC, with central role in SAC signal amplification in response to unattached kinetochores. Mad2 overexpression was reported in breast cancer cell lines and tissue samples [37,41,42]. Mad2 upregulation was found along with its functional antagonist p31\textsuperscript{comet}, and it seems that altered p31\textsuperscript{comet}:Mad2 expression ratios might lead to changes in SAC activity and the generation of CIN in breast cancer [42]. Downregulation of Mad2 was found in a mouse model deficient for BRCA-1, suggesting a role of SAC deregulation in genome instability due to mutated BRCA-1 [43]. Decreased Mad2 was also reported in a human breast tumor line sensitive to taxol and nocodazole, indicating that defects in SAC may contribute to the sensitivity of breast cancer to anti-microtubule drugs [41]. Negative Mad2 expression was associated with HER-2 overexpression in invasive ductal breast carcinoma [37]. Otherwise, no clinical implication of Mad2 expression profile for breast cancer was reported up to now.

4.6. Aurora B kinase

Aurora B is a serine/threonine protein kinase that binds to kinetochores involved in kinetochore-microtubule attachment, SAC activity, and cytokinesis [44]. Aurora B senses improper kinetochore-microtubule attachments, due to lack of tension on sister-kinetochores, and destabilizes them, which activates the SAC thus providing more time for successful chromosome biorientation [45]. Additionally, Aurora B is required for recruitment of SAC proteins to the kinetochore and, thus, is essential for SAC function. Aurora B was found to be upregulated in breast carcinoma, with strong correlation with cell proliferation but not with overall survival [28,35,46-48].
Owing to its role in cell proliferation and its oncogenic potential, Aurora B was proposed as attractive target for breast cancer therapeutics [48].

4.7. Mps1

Mps1 (monopolar spindle 1, also known as TTK) is a dual-specificity protein kinase protein kinase that binds to kinetochores. Mps1 is required to sustain SAC functions and promotes correct kinetochore-microtubule attachment [49]. High expression of Mps1 was reported in breast tumor tissues and was shown to have a protective role of aneuploidy in breast cancer cell lines [28,30,50]. In a meta-analysis using the Oncomine database, high Mps1 expression, both at mRNA and protein levels, was found to be associated with high tumor grade, high Ki67 expression, and worse survival, particularly in TNBC [51], suggesting Mps1 as an attractive therapeutic target for this aggressive subgroup of breast cancer [52].

Table 1. Expression levels of spindle assembly checkpoint components and clinical implications in breast cancer.

<table>
<thead>
<tr>
<th>SAC protein</th>
<th>Expression levels</th>
<th>Suggested clinicopathological significance</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bub1</td>
<td>Overexpression</td>
<td>High histological tumor grade</td>
<td>[30-35]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tumorigenesis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Poor metastasis-free survival</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aggressiveness</td>
<td></td>
</tr>
<tr>
<td>BubR1</td>
<td>Overexpression</td>
<td>High histological tumor grade</td>
<td>[30-33,36-39]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tumorigenesis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IHC biomarker of malignancy</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Poor survival</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Associated with Ki-67</td>
<td></td>
</tr>
<tr>
<td>Bub3</td>
<td>Overexpression</td>
<td>-</td>
<td>[28,30]</td>
</tr>
<tr>
<td>Mad1</td>
<td>Overexpression</td>
<td>-</td>
<td>[28,30,40]</td>
</tr>
<tr>
<td>Mad2</td>
<td>Overexpression</td>
<td>Aggressiveness</td>
<td>[37,41,42]</td>
</tr>
<tr>
<td></td>
<td>Underexpression</td>
<td>NA</td>
<td>[43]</td>
</tr>
<tr>
<td>Aurora B</td>
<td>Overexpression</td>
<td>High histological tumor grade</td>
<td>[28,35,46-48]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tumorigenesis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aggressiveness</td>
<td></td>
</tr>
<tr>
<td>Mps1</td>
<td>Overexpression</td>
<td>High histological tumor grade</td>
<td>[28,30,50-52]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tumorigenesis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Poor metastasis-free survival</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative correlation with overall survival</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aggressiveness</td>
<td></td>
</tr>
<tr>
<td>Cdc20</td>
<td>Overexpression</td>
<td>Tumorigenesis</td>
<td>[28,30,46,53-55]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Associated with Ki-67</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Poor prognosis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aggressiveness</td>
<td></td>
</tr>
</tbody>
</table>
Overall, overexpression of SAC components in breast cancer seems to be consensual between different reports, while underexpression is rare. The molecular mechanisms behinds this increased expression in SAC components are unclear. It was reported that core kinetochore genes are coordinately regulated downstream of the Forkhead Transcription factor FoxM1, a regulator of genes involved in late events of the cell cycle, indicating that SAC components are expressed collectively rather than individually [62]. Given the correlation with proliferation markers, overexpression of SAC components may serve to sustain proliferation of aneuploid cancer cells.

### 5. SAC AS TARGET FOR BREAST CANCER THERAPIES

Microtubule-targeting agents (MTAs) remain the most conventional yet reliable chemotherapeutic agents, particularly in breast cancer where they are used as first-line treatment. Paclitaxel (Taxol), a microtubule stabilizer, is commonly used in the treatment of metastatic breast cancers [63]. It binds neighboring tubulin dimers along the interior surface of the microtubules, thereby stabilizing tubulin polymers [64]. Chromosomes still attach to these stabilized microtubules, yet they are unable to achieve proper biorientation due to the lack of tension across sister-kinetochores. As a consequence, the cells arrest and die in mitosis due to prolonged activation of SAC [65]. Therefore, SAC activity is required for effective anti-mitotic therapies mediated by MTAs. While taxanes are commonly used in the treatment of breast, the use of microtubule destabilizer agents is still limited to investigational purpose on advanced breast cancer [66]. However, MTAs are not free of undesirable side effects: normal dividing cells are also affected, resulting in myelosuppression, and, as microtubules are required for vesicular trafficking in interphase, MTAs also elicit neurotoxicity [67]. Resistance to MTAs also limits their effectiveness [68]. Therefore, novel anti-mitotic drugs are being developed to address these side effects. In this respect, SAC has been regarded as an attractive target for anti-mitotic therapies.

Anti-mitotic activity can be achieved either by inhibiting mitotic exit or by forced mitotic exit, depending on the targeted SAC component. The rational of this approach is that: i) inhibiting mitotic exit should maximize the time tumor cells spend in mitosis, ultimately leading to their death; and, alternatively, ii) forcing tumor cells to

<table>
<thead>
<tr>
<th>SAC Component</th>
<th>Expression</th>
<th>Prognostic Factor</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plk-1</td>
<td>Overexpression</td>
<td>High histological tumor grade</td>
<td>[28,35,36,56-58]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Poor prognosis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aneuploidy</td>
<td></td>
</tr>
<tr>
<td>Hecl</td>
<td>Overexpression</td>
<td>Tumorigenesis</td>
<td>[28,51]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aggressiveness</td>
<td></td>
</tr>
<tr>
<td>CENP-E</td>
<td>Overexpression</td>
<td>Aggressiveness</td>
<td>[28,59-61]</td>
</tr>
<tr>
<td>RZZ</td>
<td>Overexpression</td>
<td>NA</td>
<td>[28]</td>
</tr>
<tr>
<td>p31comt</td>
<td>Overexpression</td>
<td>NA</td>
<td>[42]</td>
</tr>
</tbody>
</table>

NA: not available
exit mitosis prematurely should inevitably result in their death as a consequence of massive chromosome mis-segregation. Both approaches were tested in human breast cancer cells, with varying degrees of success. It should be noted that there are two functionally different groups of SAC components, that elicit two different types of response upon inhibition: i) the core SAC components (Aurora B, Mps1, Bub1, BubR1, Bub3, Mad2, and Mad1) which are involved in the generation of the diffusible inhibitory signal, and whose inhibition results in precocious mitotic exit; and ii) the SAC components (Plk1, Hec1, Mis12, KNL-1, Rod/Zwilch/Zw10, Ska1-3, CENP-E, and dynein) which are involved in kinetochore-microtubule attachment and/or chromosome congression but are not required for SAC signal generation, and whose inhibition elicits a mitotic arrest. In principle, most SAC components are potential druggable targets [15]. Some of these targets were evaluated in breast cancer cells as described below and summarized in (Table 2).

Treatment of breast cancer cells with the potent and selective inhibitor of Mps1 kinase activity, NMS-P715, caused SAC override, induced mislocalization of kinetochore components, and led to cell death as a consequence of massive aneuploidization [69]. This small molecule was tested in a large cell line panel and breast cancer cells were among the most sensitive. Another Mps1 inhibitor called reversine also resulted in cell death by apoptosis after a transient arrest of breast cancer cells in mitosis [70]. The Mps1 inhibitor SP600125 (initially known as a Jnk pathway inhibitor) reduced viability of doxorubicin-resistant breast cancer cells [71]. Altogether, these reports indicate that SAC abrogation is a promising approach to breast cancer therapy. Selective inhibition of aurora B kinase activity by AZD1152, the prodrug for AZD1152-HQPA, was reported to cause mitotic catastrophe, polyploidy and apoptosis in a panel of breast cancer cell lines, including HER2-overexpressing lines. AZD1152 administration efficiently suppressed the tumor growth in a breast cancer cell xenograft model, highlighting the antineoplastic potential of this compound for breast cancer [72]. Many reports highlighted the antineoplastic potential of Plk1 (Polo-like kinase 1), a serine/threonine kinase with multiple functions in mitosis including proper progression through multiple stages of mitosis, including mitotic entry, centrosome maturation, bipolar spindle formation, chromosome congression and segregation, cytokinesis, and mitotic exit [73]. Inhibition of Plk1 with the small molecule BI 2536 caused a mitotic arrest and induced apoptosis in breast cancer cell lines [74]. Similar effects were observed with the Plk1 inhibitors TAK-960 and NMS-P937, with significant antitumor activity and acceptable tolerability in breast cancer xenografts [75,76]. Interestingly, inhibition of Plk1 by antisense oligonucleotides was reported to potentiate the neoplastic effect of paclitaxel, causing synergistic effects in breast cancer cell lines and in human breast cancer xenografts [77]. However, while BI 2536 showed acceptable safety profile in phase I clinical trial, limited antitumor activity was observed in phase II in patients
with advanced breast cancer [78,79]. Inhibition of CENP-E, a kinetochore kinesin that functions in chromosome congression, by the small molecule GSK923295 arrested breast cancer cell in mitosis and induced cell death through apoptosis [80]. A potent CENP-E inhibitor (PF-2771) induced complete tumor regression in a patient-derived TNCB xenograft model [60]. Derivatives of INH (inhibitors of Nek2 and Hec1 binding) that target Hec1/Nek2 interaction were reported to arrest breast cancer cells in mitosis and, subsequently, to induce cell death, and to retard tumor growth in human breast cancer xenografts [81].

**Table 2. Overview of existing drugs associated with SAC targeting in breast cancer.**

<table>
<thead>
<tr>
<th>Target</th>
<th>Agent</th>
<th>Reported experimental antitumor activity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mps1</td>
<td>NMS-P715; Reversing; SP600125</td>
<td>Cell death after a transient arrest in mitosis of breast cancer cell lines</td>
<td>[69-71]</td>
</tr>
<tr>
<td>Aurora B</td>
<td>AZD1152</td>
<td>Mitotic catastrophe in breast cancer cell lines; reduction in tumor size in breast tumor xenografts in mice</td>
<td>[72]</td>
</tr>
<tr>
<td>Plk1</td>
<td>BI 2536; TAK-960; NMS-P937; antisense shonucleotides</td>
<td>Apoptosis following an arrest in mitosis of breast cancer cell lines; reduction in tumor size in breast tumor xenografts in mice; synergistic effect when combined with taxol; limited antitumor activity in phase II patients with advanced breast cancer</td>
<td>[74-79]</td>
</tr>
<tr>
<td>CENP-E</td>
<td>GSK923295; PF-2771</td>
<td>Apoptosis following an arrest in mitosis of breast cancer cell lines; complete tumor regression in a patient-derived TNCB xenograft model</td>
<td>[60,80]</td>
</tr>
<tr>
<td>Hec1</td>
<td>Derivatives of INH (inhibitors of Nek2 and Hec1 binding)</td>
<td>Cell death following an arrest in mitosis of breast cancer cell lines; reduction in tumor size in breast tumor xenografts in mice</td>
<td>[81]</td>
</tr>
</tbody>
</table>

Cells exposed to MTAs arrest in mitosis, due to chronic activation of the SAC, and then they follow different fates. Amongst other fates, some cells die in mitosis while others exit mitosis in a process called slippage. It was proposed that cell fate is determined by two competing networks that run in parallel upon exposure to MTAs, one that involves accumulation of cell death signal, and another that promotes gradual cyclin B degradation [65,82]. Then, the network that reaches first its threshold will determine the fate of the mitosis-arrested cell. For instance, if cyclin B decreases below the threshold level (that sustains the mitotic state) first, then the cell will exit mitosis (slippage). Slippage is linked to resistance to anti-mitotic agents due to impaired SAC activity in many cancers [4]. In SAC defective cancers, cyclin B degradation threshold would be readily reached which favors slippage and aneuploid cells are produced. In this regard, targeting SAC components to force premature mitotic exit could be an effective strategy to circumvent this issue as this would kill cancer cells through massive aneuploidization upstream of the two competing networks (Fig. 2)).
6. CONCLUSION AND PERSPECTIVES

The last decade of research significantly increased our understanding of the signaling pathway that governs SAC activity. As a consequence, novel SAC targets and therapeutic strategies have emerged. The ability of selective SAC silencing to inhibit proliferation of breast cancer cells in vitro and in preclinical assays represents a promising therapeutic approach. Currently, numerous small molecule drugs that inhibit Mps1, Aurora B kinase, Plk1, CENP-E, and Hec1 are being tested in preclinical and clinical studies, and some have confirmed efficacy in initial clinical trials in vivo [83,84]. Although most of the preclinical assays predicted promising antitumor activity, translating this efficacy to effective bedside treatment was rather discouraging, and has raised some skepticism as to the potential use of this new generation of anti-mitotic drugs in anticancer treatment [85]. However, and in an emerging era of pharmacogenomics, the great challenge is to identify which tumors/patients are most likely to respond to these drugs [65]. For instance, the large diversity of breast cancer subtypes, together with the vast range of diversity in the genetic background between patients, impose the implementation of pharmacogenomics testing for the assessment of drug efficacy and safety [86]. Therefore, the therapeutic value of these inhibitors greatly depends on optimizing their potency and administration, and identifying biomarkers to select patients. Also, determining the value of combining these compounds with classical microtubule-targeting drugs deserves further exploration as it might result in maximal clinical benefit with reduced toxicity. Finally, as SAC is only required during mitosis, and since mitosis represents a relevant point of intersection of numerous signaling pathways, it is now clear that SAC targeting is an appealing strategy to kill cancer cells, especially those with high rate of proliferation and chromosome instability.

ABBREVIATIONS

- **APC/C**: anaphase promoting complex or cyclosome
- **Bub**: budding uninhibited by benzimidazole
- **BRCA**: Breast cancer
- **Cenp**: centromere protein
- **Cdc20**: Cell division cycle 20
- **CDK1**: cyclin-dependent kinase 1
- **CIN**: chromosomal instability
- **ER**: estrogen receptor
- **HER**: human epidermal growth factor receptor
- **Hec1**: Highly expressed in cancer
- **Mad**: mitotic arrest deficient
- **o-Mad2**: open mitotic arrest deficient 2
**CONFLICT OF INTEREST**

The authors confirm that this article content has no conflicts of interest.

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Graphical abstract