In: Horizons in Cancer Research. Volume 64 ISBN: 978-1-53610-512-4
Editor: Hiroto S. Watanabe

Chapter 5

CHROMOSOME INSTABILITY AS A TARGET FOR CANCER THERAPEUTICS: CHALLENGES OF MITOTIC INHIBITORS

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ABSTRACT

Accurate control of chromosome segregation is crucial for genomic stability. The spindle assembly checkpoint (SAC) strictly monitors the fidelity of chromosome separation during mitosis. Once a mitotic abnormality is detected, the activated SAC blocks mitotic progression, frequently inducing apoptosis during the prolonged mitotic arrest (termed mitotic death). Thus, the SAC plays an important role in eliminating aneuploidy to maintain the fidelity of chromosome stability. Broadly recognized as a hallmark of cancer, aneuploidy, in contrast, causes imbalanced gene expression and subsequent aneuploidy-associated stresses, such as DNA damage and proteotoxicity. These stresses may be involved in a cancer-specific vulnerability to aberrant chromosome dynamics. Antimitotic therapeutics that disturb the microtubule dynamics required for mitotic spindle formation are widely used in the clinical treatment of cancer. To expand the therapeutic window of the current

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tubulin-mediated antimitotic drugs, next-generation mitotic inhibitors are being developed by various pharmaceutical and biotechnology companies. This review summarizes how chromosome segregation is accurately controlled and how SAC monitors this process to maintain segregation fidelity. The involvement of aneuploidy in tumor development and clinical treatment is also discussed. Lastly, an overview of the mitotic inhibitors in preclinical and clinical development as cancer therapeutics is provided.

INTRODUCTION

It has been over 100 years since Théodore Bovéri observed abnormal karyotypes in cancer cells [1, 2]. Today, aneuploidy, which is defined as an aberrant number of chromosomes, is recognized as a hallmark of human cancer [3-7]. The gain or loss of chromosomes in aneuploid cells causes a marked imbalance in gene expression due to aberrant gene copy numbers, drastic changes in cellular homeostasis, and a significant restructuring of novel signaling networks to counteract aneuploidy-mediated stress [8-11]. These aneuploidy-mediated signaling networks may be involved in cancer-specific phenotypes or vulnerabilities. Indeed, aneuploidy is of vital importance to clinical practice because the most advanced-stage cancers are typically comprised of aneuploid tumor cells [12]. Large-cohort clinical studies revealed a significant correlation of aneuploidy with aggressiveness, poor prognosis, and drug-resistant tumors [12-18]. Thus, aneuploidy is not only a characteristic feature of tumors in cancer biology but also a valuable indicator to predict patient prognosis and the drug response of tumors in clinical practice.

This review describes the molecular mechanisms of chromosome segregation and how the spindle assembly checkpoint (SAC) machinery maintains the fidelity of this segregation. In addition, the role of aneuploidy in cancer development, prognosis, and drug resistance is discussed. Lastly, an overview of mitotic inhibitors in preclinical and clinical development as cancer therapeutics is provided. This review may aid in the development of next-generation mitotic inhibitors to overcome the difficulties associated with the resistance of cancer cells to current antimitotic drugs.

1. THE SPINDLE ASSEMBLY CHECKPOINT MAINTAINS THE FIDELITY OF CHROMOSOME SEGREGATION

1.1. Chromosome Segregation in Mitosis

The genome is replicated during S phase, and it needs to be equally distributed into daughter cells during the division process [19]. Well-orchestrated machineries precisely control the transmission of genetic information to the next generations. The sister chromatid pairs, newly synthesized by DNA replication, are bundled with a ring structure complex of four protein subunits (cohesins) [20, 21]: SMC1, SMC3, SCC1, and SCC3 [21-24]. SMC1 and SMC3 form V-shaped dimers with ATPase domains at the ends, and the ATP-dependent interaction between these domains results in a ring structure [25]. The SCC1 and SCC3 subunits bind to the ATPase domains of SMC1 and SMC3 [26]. SCC1 helps lock the ring structure in the closed position to hold the sister chromatid pairs together until their separation at the transition from metaphase to anaphase [27]. Chromosomes structurally change during prophase to prepare for sister chromatid separation [28, 29]. During prometaphase and metaphase, the chromosomes are tightly compacted, and the arms of sister chromatids attached at the centromere emerge. By metaphase, the cohesins are mostly removed from the arms but remain concentrated at the centromere [30-32]. In mammals, the mitotic cohesin complex is phosphorylated by Polo-like kinase 1 (PLK1) to release the cohesins from chromosomes during prophase, i.e., the prophase pathway, whereas the centromeric cohesin complexes are protected in a shugoshin (SGOL1)-dependent manner [33, 34]. SGOL1 recruits protein phosphatase 2A to the centromeres to counteract PLK1-mediated cohesin complex phosphorylation. Thus, centromeric cohesion is protected from the prophase pathway in mitotic cells.

The mitotic spindle is a bipolar array of microtubules that segregates sister chromatids by pulling the equally divided chromosome sets to each pole of the cell [19, 20]. The bipolar microtubule array of a mitotic spindle is organized in four steps: microtubule nucleation [35]; formation of antiparallel microtubules cross-linked by EG5 (also called KSP or kinesin-5) [36]; kinesin-4 and -10 motors attached to the chromosome arms capture the antiparallel microtubules and push the microtubule minus-ends away from chromosomes [37]; and microtubule minus ends are focused into spindle poles. The plus-end microtubules search and capture the sister chromatid kinetochore [38], which

is a protein structure on chromatids for spindle fiber attachment [39]. Once sister chromatids achieve bi-orientation by attachment between plus-end microtubules and the kinetochore at both sides, the chromatid pairs congress at the spindle equator, i.e., the metaphase plate [20, 21, 39, 40].

After alignment at the metaphase plate, the cells are ready for sister chromatid separation toward each spindle pole [19-21, 39, 40]. Chromosome separation is triggered by activation of the protease separase, which cleaves SCC1, opens the cohesin ring structure, and releases the sister chromatid pair from the bound position [20, 21, 41, 42]. Separase activity is suppressed until the metaphase/anaphase transition of two molecules: securin and CDK1/cyclin B [20]. Securin binds tightly to separase at its active site, thus inhibiting protease activity [42]. Phosphorylation of separase by CDK1/cyclin B also inhibits protease activity [43]. The anaphase-promoting complex (APC/C) with activator subunit CDC20 (APC/C-CDC20), which is an E3 ubiquitin ligase, targets poly-ubiquitination of both securin and cyclin B to promote degradation of these proteins by the 26S proteasome [40]. Thus, APC/C-CDC20 releases separase from the inhibitory machinery at metaphase, and activated separase initiates sister chromatid separation by SCC1 cleavage.

1.2. Spindle Assembly Checkpoint Machinery

Chromosome segregation during mitosis involves a dynamic interaction between spindle microtubules and kinetochores. This interaction is required for the bipolar attachment between kinetochores and microtubules and subsequent alignment of sister chromatids to the metaphase plate [19, 40, 44]. To maintain fidelity during chromosome segregation, the SAC mechanism regulates the proper attachment of microtubules to kinetochores and the tension between the kinetochores of sister chromatids [19, 40, 44]. The incorrect attachment between the kinetochore and microtubules, such as syntelic and merotelic attachment, triggers the SAC to generate a “wait anaphase signal” that suppresses the ubiquitin ligase activity of APC/C-CDC20 and inhibits the degradation of securin and cyclin B [45, 46]. Thus, the SAC prevents the premature separation of sister chromatids until the kinetochores of each duplicated chromosome pair have achieved bipolar attachment to the mitotic spindle [19, 40, 44-46].

The SAC negatively regulates APC/C-CDC20 ubiquitin ligase activity through the mitotic checkpoint complex (MCC), which is composed of the following: MAD2, BUB1B-related protein (BUBR1), and BUB3; other SAC-

associated proteins, such as MAD1, BUB1, and MPS1; and the KNL1-MIS12-NDC80 (KMN) complex networks [44, 47]. These proteins are preferentially localized at the kinetochores of unaligned chromosomes. The most recognized hypothesis is that the MAD1-MAD2 complex acts as a sensor of unattached kinetochores [44, 48]. MAD2 is stably bound to MAD1 at the unattached kinetochores until the bi-oriented kinetochore-microtubule attachment of sister chromatids is formed with the appropriate inter-kinetochore tension [49, 50]. The MAD1-MAD2 complex at the unattached kinetochores catalyzes the binding of soluble MAD2 with CDC20, thus releasing the MAD2-CDC20 complex from the kinetochores [19, 44, 48]. The defused MAD2-CDC20 complex serves as a catalyst to generate additional MAD2-CDC20 complexes, which form the MCC by binding to BUBR1-BUB3 [19, 44, 48]. The MCCs produced by feedback loops bind to the APC/C and suppress the APC/C E3 ligase that induces the wait anaphase signal [19, 44, 48]. Thus, disruption of the SAC machinery attenuates the wait signal in response to inappropriate attachment between kinetochores and microtubules, often leading to chromosome missegregation or premature mitotic exit and aneuploidy, a hallmark of many solid tumors [3-7, 51, 52].

1.3. SAC Attenuation and Cancer

Defects in the SAC may contribute to the chromosomal instability observed in various human cancers [51, 52]. The deletion or inactivation of several SAC proteins can result in a loss of checkpoint control, premature anaphase onset, and subsequent chromosomal instability [7, 53-57]. Genetically engineered mouse models demonstrate that the reduced expression of SAC components, such as BUBR1, BUB1, MAD1, MAD2, and CENP-E, elevates the incidence rate of spontaneous cancers [2, 56, 58-61]. In human primary tumors, the protein expression of BUBR1 is significantly reduced in ~30% of colon adenocarcinomas when compared with normal colorectal tissues [62]. Immunohistochemistry also revealed low BUBR1 protein expression of in ~60% tumors [11]. A genomic analysis revealed that oncogenic mutations or the downregulation of SAC-associated genes is found in multiple cancer types, although these mutations occur less frequently in tumors [6, 7, 62]. Given that aneuploidy is frequently observed in human cancers, the SAC mechanism is presumed to be attenuated epigenetically, or genetically in some cases, in a broad spectrum of human primary tumors.

2. ANEUPLOIDY AND CANCER

Since Théodore Bovéri observed abnormal karyotypes in cancer cells over 100 years ago, aneuploidy has become recognized as a hallmark of human cancer [1, 2, 12, 63]. The gain or loss of chromosomes can result from chromosome missegregation via misassembled kinetochore protein, aberrant kinetochore-microtubule attachment, or attenuated SAC machinery [7, 64]. More than 70% of solid tumors are aneuploid, and aneuploidy correlates with the aggressiveness, poor prognosis, and drug resistance of tumors [5, 6, 12]. Indeed, large-cohort clinical studies correlated aneuploidy with poor prognosis and drug-resistant tumors [13-18]. Thus, aneuploidy is not only a characteristic feature of human cancer but also a potentially important factor in determining the prognosis and/or the therapeutic strategies in cancer patients.

2.1. Aneuploidy in Colorectal Cancer

Colorectal cancer (CRC) is the third most common malignancy in both males and females worldwide (http://www.wcrf.org/). CRC is also one of the most advanced research areas on the etiological characterization of the disease and aneuploidy [12, 65-67]. Based on the presence of repetitive DNA sequences (i.e., microsatellites), CRCs are classified into two categories [66]: microsatellite stable CRCs (MSS; ~85% of sporadic CRCs) and microsatellite instable CRCs (MSI; ~15% of sporadic CRCs) [67]. MSS substantially overlaps with chromosomal instability (CIN), which increases the rate of chromosomal gain or loss during mitosis [12, 65, 67-69]. Aneuploidy in cancer cells appears to be a result of CIN [2, 70, 71]. Although CIN CRC is a major subgroup of the CRC classification, the molecular mechanisms underlying CIN and tumor development remain to be elucidated. The potential hypotheses include genetic mutations of the SAC and DNA repair, DNA checkpoint, and telomerase pathways. Although each hypothesis may be supported by a specific preclinical model, none of these hypotheses are prominent enough to clarify the fundamental mechanisms across a broad spectrum of consistently observed CIN CRCs [68, 69]. To date, the most broadly supported hypothesis in CIN CRCs involves the adenomatous polyposis coli gene (*APC*), which shows a high frequency of truncation mutations in this cancer subgroup [72]. Mutant *APC* attenuates kinetochore-microtubule attachment and consequently generates significant aneuploidy that is enhanced by a p53 loss of function [73].

The accumulating evidence from clinical studies demonstrates that aneuploidy may be a useful indicator to predict the clinical response to cancer therapeutics [5, 6, 12]. CIN is associated with a poor prognosis. CIN CRC patients consistently exhibit low rates of overall and progression-free survival when compared with MSI CRC patients [12, 67-69, 74, 75]. In addition, the CIN CRCs were reported to be intrinsically resistant to microtubule inhibitors [76]. More recently, it was reported that the immune-checkpoint inhibitors, anti-PD1 and anti-PDL1 antibodies, are potently effective for MSI CRC but not CIN CRC, presumably because the mutation rates of the genome in CIN CRC are much less than those in MSI CRC, and CIN CRCs do not exhibit enough cell surface neoantigen for the immune-checkpoint inhibitors show antitumor efficacy [77, 78]. Thus, aneuploidy could be a useful indicator in CRCs not only to predict prognosis but also to determine the therapeutic strategy.

2.2. Aneuploidy in Ovarian Cancer

Ovarian cancer is one of the most common and aggressive gynecological malignancies (http://www.wcrf.org/): the fifth most lethal in all women and the most lethal of all gynecological malignancies (~50% at five year survival rate) [79]. A number of studies have demonstrated that advanced ovarian cancers exhibit aneuploidy at a highly frequent rate, and DNA polyploidy is highly correlated with the prognosis of ovarian cancers [80]. Aneuploidy is significantly associated with cancer stage and cancer grade, and the patients with aneuploid tumors have significantly shorter survival rates than the patients with diploid tumors [80].

Based on their molecular and histopathologic features, ovarian cancers are categorized in two major groups: type-I and type-II [15, 79, 81]. Type-I ovarian cancers are comprised of low-grade serous borderline tumors, clear cell tumors, endometrioid tumors, and mucinous tumors [15, 79, 81]. In contrast, type-II ovarian cancers are more aggressive and comprise the high-grade serous tumors, high-grade endometrioid tumors, carcinosarcomas, and undifferentiated carcinomas [15, 79, 81]. The majority of type-II ovarian cancers carry TP53 mutations, and ~50% of these exhibit BRCA1/2 dysfunction either genetically or epigenetically due to a homologous recombination defect (HRD). Thus, CIN and aneuploidy are hallmarks of type-II ovarian cancers [15, 79-81]. Given that the high frequency of HRDs is observed in type-II aneuploid ovarian cancers, these cancers are expected to be

more sensitive to poly(ADP-ribose) polymerase (PARP) inhibitors, which produce synthetic legality in HRD cancers [82-87]. Thus, the screening for the aneuploidy phenotype may help determine the appropriate clinical treatment strategy for ovarian cancers.

2.3. Aneuploidy in Gastric Cancer

Gastric cancer is the fifth most common cancer worldwide (http://www.wcrf.org/) and exhibits a high frequency of aneuploidy (24%–96%) [18]. Aneuploidy, which is detectable in primary tumors by flow cytometry, has been demonstrated to be a useful prognostic marker in gastric cancers. Indeed, aneuploid gastric cancers exhibit high proliferative activity, increased metastatic potential, and poor prognosis when compared with diploid gastric cancers [17, 18, 88]. Similar to CRC, MSI is also found in ~20% of gastric cancer patients [18, 89-91], and aneuploid gastric tumors are well correlated with MSS cancers [92]. Similar to CRCs, aneuploid gastric cancers are expected to show a diminished clinical response to immune-checkpoint inhibitors, suggesting that different therapeutic strategies should be used for aneuploid-driven gastric cancers [93-95]. A deeper understanding of the effects of aneuploidy on cellular homeostasis will aid in the development of novel cancer therapeutic drugs that target aneuploid cancer cells.

3. CHROMOSOME DYNAMICS AND CANCER THERAPEUTICS

The gain and loss of chromosomes in aneuploid cancer cells produces imbalanced gene expression due to a fractionation of gene copy numbers [8, 9]. Imbalanced gene expression can affect various cellular functions and signaling pathways, such as the unfolded protein response and DNA replication stress [8-11]. Notably, aneuploid cancer cells appear to chronically suffer from these aneuploid-associated stresses. Thus, understanding the effects of these stresses on cancer survival signaling may provide clues for cancer therapeutic strategies that target cancer-specific vulnerabilities. One approach is to enhance aneuploid-associated stresses with mitotic inhibitors. In fact, mitotic inhibitors, such as paclitaxel and vinca alkaloids, are widely used as standard cancer treatments [96-98].

Mitotic inhibitors are classified into two major categories: microtubule-targeting agents and molecular-targeting inhibitors of mitotic protein components [96-101]. The microtubule-targeting agents, which include taxanes and vinca alkaloids, target microtubule dynamics and interfere with microtubule polymerization and depolymerization, which leads to abnormal spindle formation and chromosome misalignment [96-98]. In contrast, molecular-target inhibitors target the molecular components that regulate chromosome dynamics and mitotic spindle formation [96, 98-102]. Microtubule inhibitors are widely used in the clinical treatment of cancer; however, peripheral neuropathy is a major adverse effect of these drugs, presumably because they directly inhibit the assembly of microtubule structures even in non-dividing neural cells [96-98, 103, 104]. To reduce the incidence of this debilitating side effect, the regulatory components of mitotic spindles and chromosome dynamics that are non-structural but essential for mitosis have recently attracted attention as target molecules for next-generation anticancer drugs [96, 101, 102, 105].

3.1. Microtubule-Targeting Agents

Microtubule-targeting agents are one of the most classical and reliable set of chemotherapeutics that have been widely used in the clinical treatment of cancer [96-98]. Microtubule-targeting agents disrupt proper microtubule dynamics, which leads to abnormal spindle formation, chromosome misalignment, and SAC-induced prolonged mitotic arrest [96-101]. Although the detailed mechanisms of these drugs on anti-proliferative activity remain unclear, prolonged mitotic arrest appears to be a central mechanism that induces cell death in tumors. Microtubule-targeting agents are classified into two categories based on their mechanism of action: microtubule-stabilizing agents and microtubule-destabilizing agents [96-98]. Drugs in both categories exhibit potent antiproliferative activity in a broad range of preclinical cancer models and are clinically used as a standard chemotherapeutic drugs for the treatment of various cancer types [96-101].

3.2. Microtubule-Stabilizing Agents

Microtubule-stabilizing agents, including taxanes (paclitaxel [103] and docetaxel [104]) and epothilone (ixabepilone) [106], interact with the β-tubulin

of microtubules to decrease the dissociation of β-tubulin from the neighboring α-tubulin. This prevents microtubule depolymerization, which leads to abnormal microtubule dynamics during mitotic spindle formation [96-98]. Although the attachment of kinetochores and stabilized microtubules can still be established, microtubule-stabilizing agents perturb the inter-kinetochore tension of sister chromatids via improper bi-orientation [96-101]. Microtubule-stabilizing agents are used as a standard cancer treatment for a broad range of solid tumors [96-101].

1) **Paclitaxel** [103]: Paclitaxel, which is extracted from the Pacific yew tree *Taxus brevifolia*, was discovered as a compound with antiproliferative activity. Paclitaxel is approved as a treatment for various tumors, such as ovarian, breast, lung, and pancreatic cancers.
2) **Docetaxel** [104]: Docetaxel is a semi-synthetic analog of paclitaxel derived from a compound found in the European yew tree *Taxus baccata*. Docetaxel is approved as a treatment for various tumors, such as breast, gastric, lung, prostate, and head and neck cancers.
3) **Ixabepilone** [106]: Ixabepilone is an orally bioavailable semisynthetic analog of epothilone B with antiproliferative activity. Ixabepilone binds to tubulin and stabilizes microtubules, and it is an approved treatment for breast cancer.

3.3. Microtubule-Destabilizing Agents

Microtubule-destabilizing agents, such as vinblastine and vincristine, bind to β-tubulin in the vinca domain, a region adjacent to the GTP-binding site, which produces conformational changes and microtubule destabilization [97, 98]. Microtubule-destabilizing agents also induce aberrant mitotic spindle formation and misaligned chromosomes during metaphase, and subsequent SAC activation causes prolonged mitotic arrest and cell death [97, 98]. These agents are clinically used in combination with other chemotherapeutic drugs for hematological tumors and several types of solid tumors [97, 98].

1) **Vincristine**: Vincristine is a natural alkaloid isolated from the plant *Vinca rosea* Linn. Vincristine is an approved treatment for hematological cancers, solid tumors, and sarcomas, such as acute leukemia, Hodgkin and Non-Hodgkin lymphomas, neuroblastomas, rhabdomyosarcomas, and Wilms tumors.

2) **Vinblastine**: Vinblastine is a natural alkaloid isolated from the plant Catharanthus roseus. Vinblastine is an approved treatment for various tumors, such breast cancer, testicular cancer, choriocarcinoma, Hodgkin and Non Hodgkin lymphoma, Kaposi sarcoma, and mycosis fungoides.
3) **Vinorelbine**: Vinorelbine is a semisynthetic vinca alkaloid isolated from the plant *Vinca rosea*. Vinorelbine is an approved treatment for lung cancer.

3.4. Side Effects of Microtubule Inhibitors

Microtubule inhibitors potently exert antitumor effects on proliferating cancer cells; however, they also induce microtubule disorders in non-proliferating cells, including the disruption of vesicular trafficking, axonal transport deficits, and disorganized cytoskeleton dynamics [96-98, 103, 104]. Peripheral neuropathy, which is a major side effect of microtubule inhibitors, may be derived from microtubule disorders in peripheral neuronal cells, presumably because the microtubule inhibitors directly inhibit microtubule structures, even in non-dividing neural cells [96-98, 103, 104]. To reduce this side effect, non-structural components of microtubules, such as aurora kinases, polo kinases, and mitotic kinesins, have recently attracted attention as novel targets of next-generation cancer therapies [96-102].

3.5. Target Molecule Inhibitors of Mitotic Components

3.5.1. Aurora Kinase Inhibitors

Aurora kinases are serine/threonine kinases that play multiple essential roles in chromosome segregation control [7, 102, 107, 108]. Three Aurora kinases have been identified in mammalian cells: Aurora A, B, and C [108]. Given their elevated expression profiles in many human cancers, Aurora A and B kinases are potential targets for novel small-molecule inhibitors [7, 102, 107-109]. The molecular functions of these kinases during mitosis are distinct. Aurora A regulates centrosome maturation and separation during the early stages of mitosis, whereas Aurora B is involved with kinetochore-microtubule attachment and cytokinesis during the later stages of mitosis. Several small molecule inhibitors targeting Aurora A and/or Aurora B have been developed

and are being evaluated for their antitumor activity in clinical trials [96, 100, 102, 108-111].

1) **Alisertib** [112]: Alisertib (MLN8237) is an orally available, selective Aurora A inhibitor with an IC50 of 1.2 nM and more than a 200-fold greater selectivity for Aurora A over Aurora B. Phase 3 clinical trials are ongoing.
2) **Barasertib** [113]: Barasertib (AZD1152) is a selective Aurora B inhibitor with an IC50 of 0.37 nM and more than 3000-fold greater selectivity for Aurora B over Aurora A. Phase 2 clinical trials are ongoing.
3) **Tozasertib** [114]: Tozasertib (VX680/MK-0475) is a first generation pan-Aurora kinase inhibitor with an IC50 of 0.6 nM, 18 nM, and 4.6 nM against Aurora A, B, and C, respectively. Phase 2 clinical trials were discontinued.
4) **Danusertib** [115]: Danusertib (PHA-739358) is a pan-Aurora kinase inhibitor with an IC50 of 13 nM, 79 nM, and 61 nM against Aurora A, B, and C, respectively. Phase 2 clinical trials are ongoing.
5) **PF-03814735** [116]: PF-03814735 is a reversible dual Aurora A/B inhibitor with an IC50 of 0.8 nM and 5 nM against Aurora A and B, respectively. Phase 1 clinical trials have been completed.
6) **GSK1070916** [117]: GSK1070916 is a reversible and ATP-competitive Aurora B/C dual inhibitor with an IC50 of 3.5 nM and 6.5 nM against Aurora B and C, respectively. GSK1070916 exhibits more than 100-fold greater selectivity for Aurora B and C over Aurora A. Phase 1 clinical trials have been completed.

3.5.2. Polo-Like Kinase (PLK) Inhibitors

PLKs are a family of four serine/threonine protein kinases that are critical regulators of cell cycle progression, mitosis, cytokinesis, and the DNA damage response [118]. Four members of the PLK family (PLK1, PLK2, PLK3, and PLK4) have been identified in mammalian cells [118]. Of these, PLK1 is the most extensively studied for its molecular functions and as a cancer therapeutic drug target [96-102, 119, 120]. PLK1 plays multiple critical roles in genetic stability; spindle assembly, centrosome maturation, SAC activation, chromosome segregation, and cytokinesis [118]. Furthermore, PLK1 is upregulated in a broad range of cancer types, including ovarian, bladder, gastric, breast, colon, head and neck, esophageal, thyroid cancers, melanomas, and gliomas [102, 120]. Overexpression of PLK1 correlated with poor

prognosis [121]. Based on the potential of PLK1 as a cancer drug target, many pharmaceutical and biotechnology companies have developed small molecule inhibitors of PLK1. The antitumor activity of these compounds is being evaluated in clinical trials [96, 99, 100, 102, 122].

1) **BI2536** [123, 124]: BI2536 is an ATP-competitive PLK1 inhibitor with an IC50 of 0.83 nM and a 4- and 11-fold greater selectivity for PLK1 over PLK2 and PLK3. Phase 2 clinical trials have been completed.
2) **Volasertib** [125]: Volasertib (BI6727) is an ATP-competitive PLK1 inhibitor with an IC50 of 0.87 nM and a 6- and 65-fold greater selectivity for PLK1 over PLK2 and PLK3. Phase 3 clinical trials are being conducted for hematological cancer.
3) **Rigosertib** [126]: Rigosertib (ON-01910) is a non-ATP-competitive PLK1 inhibitor with an IC50 of 9 nM in a cell-free assay. Rigosertib also inhibits PDGFR, ABL, FLT1, CDK2, PLK2, Src, and Fyn. Phase 3 clinical trials are being conducted for hematological cancer, MDS, and pancreatic cancer.
4) **GSK461364** [127]: GSK461364 is an ATP-competitive PLK1 inhibitor with an IC50 of 2 nM (Ki) in a cell-free assay and more than a 400-fold selectivity for PLK1 over PLK2/3. Phase 1 clinical trials have been completed.
5) **MNS-P937** [128]: NMS-P937 is an orally available, highly selective ATP-competitive PLK1 inhibitor with an IC50 of 2 nM and a 5000-fold selectivity for PLK1 over PLK2/PLK3. Phase 1 clinical trials have been completed.

3.5.3. EG5 Inhibitors

The mitotic kinesin EG5 (also known as kinesin spindle protein; KSP) is emerging as a promising target for anticancer drugs [105]. EG5 is a plus-end-directed mitotic spindle motor protein of the kinesin superfamily [129] and regulates centrosome separation and bipolar mitotic spindle formation [130, 131]. Inhibition of EG5 motor activity by small molecule inhibitors causes monopolar spindle formation, SAC activation, and prolonged mitotic arrest [130, 131]. A number of small-molecule EG5 inhibitors have been or are being evaluated in clinical trials. The most advanced EG5 inhibitor is ispinesib, which has progressed to Phase 2 clinical trials [7, 96, 99-101, 105].

1) **Ispinesib** [131]: Ispinesib (SB-715992) is an allosteric EG5 specific inhibitor with an IC50 of 1.7 nM in a cell-free assay. Phase 2 clinical trials have been completed.
2) **SB-743921** [132]: SB-743921 is an EG5 specific inhibitor with an IC50 of 0.1 nM in a cell-free assay. SB-743921 has improved potency over ispinesib in both biochemical and cellular assays. Phase 1 and 2 clinical trials have been completed.
3) **Filanesib** [133]: Filanesib (ARRY-520) is a highly selective EG5 inhibitor. Phase 2 clinical trials have been completed. Phase 2 clinical trials targeting multiple myelomas are ongoing.
4) **Litronesib** [134]: Litronesib (LY2523355) is a selective EG5 inhibitor. Phase 2 clinical trials have been completed.
5) **AZD4877** [135]: AZD4877 is a potent EG5 inhibitor. Phase 2 clinical trials have been completed.
6) **ARQ-621** [136]: ARQ-621 is an allosteric EG5 specific inhibitor. Phase 1 clinical trials have been completed.
7) **MK-0731** [137]: MK-0731 is an allosteric EG5 specific inhibitor with an IC50 of 2.2 nM and more than a 20,000-fold selectivity for EG5 compared with other kinesins. Phase 1 clinical trials have been completed.
8) **EMD-534085** [138]: EMD-534085 is a potent EG5 inhibitor with an IC50 of 6 nM. Phase 1 clinical trials have been completed.

3.5.4. Centromere-Associated Protein-E (CENP-E) Inhibitors

CENP-E is another targeted mitotic kinesin for cancer therapeutic development. CENP-E controls chromosome alignment during metaphase by capturing the microtubule plus-end at the kinetochore using its ATPase motor domain [96, 99-101, 105, 129]. Recently, CENP-E was reported to transport the pole-proximal chromosomes toward the metaphase plate, and CENP-E-driven chromosome congression is guided by tubulin post-translational modification [139, 140]. Inhibition of CENP-E motor activity by small molecule inhibitors causes chromosome misalignment during metaphase, leading to SAC-mediated prolonged mitotic arrest and cell death [11, 141, 142]. In preclinical studies, CENP-E inhibitors exhibited potent antiproliferative activities in multiple xenograft mouse models and a broad range of in vitro cancer cell line models [11, 141-143]. These findings suggest that small molecules targeting the CENP-E motor domain are potential targets for anticancer drugs. However, considering that it is difficult to achieve chemical optimization with adequate pharmaceutical potency, only one CENP-

E small-molecule inhibitor, GSK923295, has been evaluated in Phase 1 clinical trials. Peripheral neuropathy, one of the major adverse effects of tubulin binders, such as taxanes or vinca alkaloids [96-98, 103, 104], was not evident in the clinical trial of GSK923295 [144]. The results of the present study demonstrate that the use of CENP-E inhibitors as anticancer drugs could potentially avoid the peripheral neuropathy associated with tubulin-binding chemotherapeutic agents [144]. This effect may be due to the selectivity of CENP-E inhibitors for proliferating cells over non-proliferating peripheral neuronal cells.

1) **GSK923295** [141]: GSK923295 is a first-in-class, allosteric CENP-E-specific inhibitor with an IC50 of 3.2 nM. Phase 1 clinical trials have been completed.
2) **PF-2771** [143]: PF-2771 is a potent, selective inhibitor of CENP-E with an IC50 of 16 nM. PF-2771 is in the preclinical phase of testing.
3) **Compound-A** [11, 142]: Cmpd-A is a time-dependent CENP-E specific inhibitor with an IC50 of 2.2 nM in a cell free assay. Cmpd-A is in the preclinical phase of testing.

CONCLUSION

In summary, accumulating evidence indicates that aneuploidy is a key biological event in tumor development and one of the most characteristic features of human cancers. In addition, aneuploidy is a potentially important indicator of the prognosis and clinical response of cancer patients. A deeper understanding of the effects of aneuploidy-mediated stresses on cellular homeostasis in cancer cells will not only significantly improve cancer therapeutic strategies but also lead to innovative drug discovery and a cure for cancers.

Disclosure of Potential Conflict of Interest

The author is an employee of Takeda Pharmaceutical Company, Ltd.

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