Targeting mitotic pathways for endocrine-related cancer therapeutics

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Abstract

A colossal amount of basic research over the past few decades has provided unprecedented insights into the highly complex process of cell division. There is an ever-expanding catalog of proteins that orchestrate, participate and coordinate in the exquisite processes of spindle formation, chromosome dynamics and the formation and regulation of kinetochore microtubule attachments. Use of classical microtubule poisons has still been widely and often successfully used to combat a variety of cancers, but their non-selective interference in other crucial physiologic processes necessitate the identification of novel druggable components specific to the cell cycle/division pathway. Considering cell cycle deregulation, unscheduled proliferation, genomic instability and chromosomal instability as a hallmark of tumor cells, there lies an enormous untapped terrain that needs to be unearthed before a drug can pave its way from bench to bedside. This review attempts to systematically summarize the advances made in this context so far with an emphasis on endocrine-related cancers and the avenues for future progress to target mitotic mechanisms in an effort to combat these dreadful cancers.

Key Words

- ▶ microtubules
- ▶ mitosis
- ▶ spindle
- ▶ endocrine
- ▶ cancer
- ▶ therapeutics
- ▶ kinetochores

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Cytoskeleton: structure and dynamics of microtubules

The human cytoskeleton plays a crucial role in maintaining cellular shape, motility of the cell as a whole and motility of organelles within the cell. Three discrete components make up the cytoskeleton: microfilaments (6nm, actin), microtubules (25nm) and intermediate filaments (10nm, keratin, vimentin, lamin, desmin, etc.) (Fletcher & Mullins 2010). A common feature these components share is that they are made up of repeating, self-assembling and disassembling building blocks called as subunits, culminating in highly dynamic filamentous structural networks necessary for a diverse array of biological functions including cell growth, rapid cell division (cytokinesis), chromosomal segregation, ciliary/flagellar movements, intracellular vesicular transport and uptake of material and signals from extracellular milieu

(Nogales 2001). This review aims to focus on microtubules as a cytoskeletal component, its allied roles in mitosis and the key conceptual advances in the field over this period, with a spotlight on its impact on the field of cancer therapeutics.

Microtubules (MTs) are polarized long hollow cylindrical structures comprising of α - and β -tubulin heterodimers. These heterodimers of 50kDa each share 50% identity at amino acid level, assemble in a head-to-tail fashion in a reversible non-covalent manner to generate protofilament; 13 of such protofilaments associate longitudinally and close up to form a MT (Akhmanova & Steinmetz 2015). These structures are highly organized yet dynamic i.e. their ends constantly experience a lengthening (polymerization) and a

shortening (depolymerization) process (Desai & Mitchison 1997). This process termed as 'dynamic instability' is highly regulated and is governed by the nucleotide-binding ability of tubulin dimers facilitating an active GTP/GDP exchange (Mitchison & Kirschner 1984). The α -tubulin binds to GTP acquiring a conformation amenable to MT polymerization, whereas β -tubulin can bind either to GTP or GDP favoring MT polymerization or depolymerization, respectively (Alushin *et al.* 2014). The MT end can be distinguished as plus (+) end if the MT is terminated by a β -tubulin protein or a minus (–) end when the MT closes with an α -tubulin subunit. Therefore, each end has a distinct characteristic that imparts structural and kinetic polarity and determines the stability. Tubulin addition and removal are much faster at the plus end than at the microtubule minus end, which is embedded in the centrosome or microtubule organizing center (MTOC) (Tran *et al.* 1997). A second form of dynamic behavior exhibited by the microtubules is 'treadmilling', which implies net growth at MT plus end and net shortening at the minus end (Margolis & Wilson 1978).

In humans, MTs exist in combinations of at least thirteen α - and β -tubulin isotypes (encoded by different genes) displaying specific cell, tissue and developmental distribution (Cleveland & Sullivan 1985). These isotypes are classified based on their high degree of sequence divergence at the carboxy (C)-termini (15–20 amino acids), which interestingly are also the hot-spots for the binding of microtubule-associated proteins (MAPs) and extensive post-translational modifications (PTMs, acetylation, tyrosination/detyrosination, poly/de-glutamylation, polyglycylation, phosphorylation, palmitoylation). This confers further chemical diversity, variability and unique functionality to each isotype (Janke & Bulinski 2011). It is well established that both these aspects (PTMs and MAPs) significantly modulate MT dynamics (Sirajuddin *et al.* 2014).

Janus with two faces: tubulin, both as a cause of and a cure for cancer

MTs are key to the success of mitosis, one of the most dramatic and crucial cellular functions. Mitosis entails effective and bonafide partitioning of replicated chromosomes among the daughter cells. In interphase, MTs exchange their tubulin with the soluble tubulin pool relatively slower (half time of several min to hours) compared to the half time of 10–30s during mitosis (Saxton *et al.* 1984). At the onset of mitosis, the

interphase MT array completely disassembles and is supplanted by a network of remodeled bipolar spindle, which is 10- to 100-fold more dynamic (characterized by both dynamic instability and treadmilling) than the MTs of interphase cytoskeleton (Rusan *et al.* 2001). After the nuclear envelope breakdown, MT dynamics is fundamental to the attachment of chromosome to the spindles in prometaphase, movement of chromosomes to the metaphase plate followed by their proper alignment and synchronous chromosomal segregation in anaphase (Mitchison 1988). Thus, to successfully locate every unattached chromosome, MTs undertake several extensive excursions, growing long distances, followed by shortening and then re-growing until they successfully establish stable connection with the chromosomes (Hayden *et al.* 1990). Considering MTs to be the key machinery driving the entire mitotic process, drugs that bind tubulin, referred to as 'tubulin-binding agents' (TBA) or microtubule targeting agents (MTA) represent soft target for the development of anti-cancer drugs (Mukhtar *et al.* 2014). Overall, one class of inhibitors operate by sequestering tubulin monomers and thus inhibiting polymerization of tubulin to form MTs. Colchicine analogs and vinca alkaloids (vinblastine, vincristine, vinorelbine, vindesine, vinflunine) are such polymerization inhibitors. The other class of inhibitors like paclitaxel analogs (docitaxel/taxotere, epithilones, discodermolide and eleutherobins) block the depolymerization of polymerized tubulin, function as microtubule-stabilizing agents and are called depolymerization inhibitors (Jordan 2002). These drugs are extremely successful and promising as chemotherapeutic drugs for combating both hematopoietic and solid tumors (Zelnak 2007). Although the mechanism of action of these mitotic poisons in each category and even within the category differ in terms of their binding site and mode of action (Mukhtar *et al.* 2014), at the cellular level, both these classes of drugs exert their effect by inducing mitotic arrest and cell death (Mollinedo & Gajate 2003).

Although MTs serve as spectacular target for various cancer treatments, there is an interesting second face to this fact. A multitude of diverse alterations in tubulin/MTs have been identified and exhaustively characterized as the cause for different varieties of cancers. This is generally attributed to aberrant expression of tubulin isotypes, erroneous post-translational modification of tubulin or differential expression of MAPs. α -Tubulin acetylation and detyrosination (by tubulin carboxypeptidase, TCP) are increased in breast cancer cell lines and tissues leading to tumor aggressiveness and poor prognosis

in patients (Boggs *et al.* 2015). TTL (tyrosine ligase), an enzyme responsible for re-tyrosination of tubulin, was observed to be suppressed during tumor growth in mice (Lafanechere *et al.* 1998). In fact, various PTMs in tubulin are correlated with tumor growth and enhanced metastasis including prostate and pancreatic cancers (Soucek *et al.* 2006, Kashiwaya *et al.* 2010, Wasyluk *et al.* 2010, Castro-Castro *et al.* 2012, Maiato *et al.* 2017). Because of their correlation with cancer, targeting PTMs represent a viable approach for targeting these altered cancer cells. Indeed, compounds such as parthenolide (an inhibitor of TCP (Fonrose *et al.* 2007)) and its more soluble derivative suitable for oral and plasma use, DMAPT, have yielded moderate success in clinical trials either alone or in combination with taxanes or vinca alkaloids (Curry *et al.* 2004, Sweeney *et al.* 2005, Shanmugam *et al.* 2010, Ghantous *et al.* 2013).

Interestingly, these alterations not only contribute to tumorigenesis but also function as another major mechanism underlying non-responsiveness of cancer cells to MTAs, besides the intrinsic or acquired drug resistance involving overexpression of drug-efflux pumps (Kavallaris *et al.* 2001). The overexpression of β III- and β IVa-tubulin isotype has been correlated with ovarian and prostate cancers and at the same time these tumors were resistant to commonly used MT-targeting therapeutics (Ranganathan *et al.* 1996, Kavallaris *et al.* 1997, Dozier *et al.* 2003, Mozzetti *et al.* 2005). Similarly, differential expression of MAPs has also been documented to interfere with MTAs; for example, overexpression of a MT-stabilizing protein MAP4 was found to enhance MT stability and counteract the MT-destabilizing drugs such as vinca alkaloids (Kavallaris *et al.* 2001).

Distinct from the α and β subunits of tubulin that compose the MTs, there is a third type of tubulin called as γ -tubulin, which in association with other proteins form a conical shaped structure, γ -tubulin ring complex (γ -TuRC) (Oakley *et al.* 2015). This complex not only provides a scaffold or template for α/β tubulin dimers during the nucleation process but also caps the (–) end while the MT continues to grow from its (+) end. This cap provides both stability and protection to the MT (–) end from depolymerizing enzymes, simultaneously inhibiting the (–) end growth (Job *et al.* 2003). BRCA1 (breast cancer 1 gene) is an E3-ubiquitin ligase that ubiquitinates Lys48 in γ -tubulin and inhibits MT nucleation thereby regulating centrosome number in cells (Sankaran *et al.* 2005). BRCA1 is breast- and ovary-specific tumor suppressor gene and its expression is reduced in the majority (55%) of sporadic epithelial ovarian cancers (EOCs), which is the most

common type representing approximately 90% of ovarian cancers (Sun *et al.* 2013). In serous ovarian carcinomas, which is a sub-category constituting about 2/3rd of EOCs, low BRCA1 expression occurs in more than 50% of cases (McMillen *et al.* 2012). Thus, in the event of loss of BRCA1 in breast cancers, centrosomes undergo hypertrophy leading to aneuploidy (Lingle *et al.* 1998). BRCA1 also influences sensitivity of breast cancer cells to MTAs, for instance, a BRCA1 mutant cell line is more sensitive to vinorelbine, a vinca alkaloid, compared to the cell lines with wild-type allele (Tassone *et al.* 2005).

Thus, in light of these issues, to combat refractory tumors and to outwit the multifactorial drug resistance, there is a pressing need to either (a) intensively search for and design new pharmacological small compounds that can bind to other domains within the tubulin by utilizing advancements in crystallographic techniques or (b) to identify new targets besides microtubules/tubulin system, an open avenue that merits further exploration. Since targeting the ubiquitously essential targets like tubulin will dampen the therapeutics window dramatically, the next generation of therapeutics should capitalize on targeting the components unique to the oncogenic cells or exclusive pathways that are either active or defective in the cancer cells such that the healthy cells are unaffected and effects can be exacerbated in the targeted cancerous cells. Examples of such targets and their contribution in the allied cellular processes are shown in Fig. 1.

Unexplored arenas for potential development of novel cancer therapeutics

Kinetochore–microtubule (kMT) interface

One of the most striking features of cancer cells is chromosomal abnormalities referred to as aneuploidy, resulting from an erosion of mitotic fidelity leading to chromosomal instability (CIN). Aneuploidy and CIN both are attributed to poor patient prognosis, metastasis and resistance to chemotherapies (Thompson & Compton 2011). Therefore, as described previously, the process of cell division logically and rationally represents an extraordinary target to develop antitumor therapies. Indeed, myriad of clinically relevant anti-tubulin drugs have proven quite effective against a wide range of tumors. However, they not only suffer drug resistance but also collateral effects, such as myelosuppression and MT disruption in non-dividing tissues, including brain (Jordan & Wilson 2004). Consequently, the kinetochores (KTs) and the peripheral proteins that participate in the

Figure 1

A schematic representation of cell cycle progression and the crucial components that are currently targeted at the various stages or can be exploited in the future for anti-cancer therapy. (1) The ORC, Cdc6 and Cdt1 assemble to form the pre-replicative complex (pre-RC) necessary to load the presumptive MCM replicative helicase, a process called as replication licensing. From late mitotic phase (M) to G1 phase, two critical inhibitors of the pre-RC formation, Cdk and geminin are suppressed by APC/C ubiquitin ligase that targets them for proteolysis through polyubiquitination (Fujita 2006). At the onset of S-phase, Cdk becomes active (by APC/C inactivation) and functions to obliterate the re-establishment of pre-RC and re-licensing during the S, G2 and M phases of the cell cycle (Fujita 2006). This is accomplished by Cdk-mediated phosphorylation of Cdc6 followed by its nuclear export, phosphorylation and degradation of ORC and Cdt1 (Fujita 2006). After S-phase, geminin also accumulates that sequesters Cdt1 by direct binding. Cdt1 however re-accumulates post G2/M-phase in phosphorylated state where it contributes in maintaining robust kMT attachments via its interaction with the Hec1 loop. (2) The kinetochore proteins start to assemble through the recognition of centromeres enriched with the CENP-A (centromere-associated protein-A) containing nucleosomes that marks it as the site for kinetochore formation. In early prometaphase or prophase, the KMN network, especially the Knl1 protein along with Zwint-1 serves to recruit several checkpoint proteins like Mad1, Mad2, Mps1 kinase, Bub1, Bub3, the RZZ complex, BubR1 and CENP-E to the unattached kinetochores for rapid generation of 'wait-anaphase' signal until every single KT pair achieves bi-orientation. This active SAC ('on-state') inhibits the anaphase-promoting complex/cyclosome (APC/C) activator Cdc20 (cell-division-cycle 20). Note that Ska binds to the Hec1 loop and its phosphorylation by Aurora B kinase subsequently abrogates its interaction with the KMN network. (3) Upon appropriate error-free KMT end-on attachments, Aurora B activates Plk1 that induces PP2A binding to BUBR1 causing the dephosphorylation of the RVSF/SILK motifs, thereby allowing PP1 to bind and act on Knl1. The binding of PP1 dephosphorylates the MELT repeats and the SAC components are released from the KMN network, extinguishing the SAC to an 'off' state. Microtubules and its associated proteins like MAPs and motor proteins are depicted. (4) After the SAC is satisfied and its components are removed from the kinetochore, APC/C regains its activity to degrade securin and cyclin B1 that triggers anaphase and exit from mitosis. Separase, a protease that cleaves the cohesins that hold sister chromatids together, is inhibited by binding to securin. But upon securin degradation, separase is released to cleave cohesin and allow the sister chromatids to undergo separation in anaphase.

mitotic process stand as an attractive therapeutic target in light of their central role in orchestrating faithful chromosome segregation. Advances in high-resolution imaging, proteomics and gene-silencing techniques have nurtured our understanding in this complex physiological

process to a great extent. Since the research in our laboratory is directed toward unraveling the underlying biological and mechanistic nuances of kinetochore-microtubule (kMT) attachment during mitosis, this review will comprehensively analyze and re-focus on a seemingly

old yet completely untapped concept of possibility and potential of targeting the kMT attachments. The kMT attachments are known to be de-regulated in tumor cells and interfering with this process is speculated to drive massive chromosomal missegregation and tumor cell death (Janssen & Medema 2013).

Kinetochores are mega-molecular multi-protein assemblies built upon centromeres and are involved in coupling the centromeric DNA to the plus ends of spindle microtubules. Some of the key components of the outer kinetochores are the protein complexes of the KMN (abbreviated for the Knl1 complex, the Mis12 complex and the Ndc80 complex) network that associates with KTs during prophase and disappears in telophase. The KMN network represents the major interface for kMT attachments (Cheeseman *et al.* 2006, DeLuca *et al.* 2006, Tooley & Stukenberg 2011), and these attachments are regulated by mitotic kinase Aurora B (ABK) (Biggins & Murray 2001, Tanaka *et al.* 2002). At the commencement of mitosis, kinetochores lacking spatial organization bind MTs indiscriminately leading to syntelic (sister KT pair attached to spindle arising from same pole) or merotelic (one KT is simultaneously attached to both the spindles emanating from opposite poles) attachments (Krenn & Musacchio 2015). At this point in early mitosis, it is imperative to keep the attachments labile and unstable so that the improperly attached MTs can be released and corrected. ABK imparts fidelity to this process by phosphorylating several kinetochore proteins including the members of the KMN network and thus weakens such erroneous kMT configurations (Welburn *et al.* 2010). However, with the progression of mitosis, stable attachments are a prerequisite to generate tension for chromosome movement. To accomplish this, there is a decline in the ABK activity and a concomitant increase in kinetochore phosphatase activity, which dephosphorylates the Ndc80 complex facilitating the formation of stable MT attachments (Liu *et al.* 2010). Therefore, a defective ABK regulatory system results in erroneous kMT attachments leading to chromosomal missegregation and chromosomal instability (CIN), a hallmark of cancer. In fact, cancer cells with CIN possess an inherently reduced capacity to correct erroneous kMT attachments and especially merotelically (Bakhom *et al.* 2009a,b, Bakhom & Compton 2012).

Highly expressed in cancer protein 1 (Hec1), a constituent of the evolutionary conserved Ndc80 complex of the KMN network directly bridges the KTs with MTs. As the name suggests, this protein is

frequently overexpressed in cancer cells (Wu *et al.* 2008). Consequently, Hec1 depletion in tumor cell lines and in xenografts induced mitotic abnormalities and cell death (Gurzov & Izquierdo 2006, Li *et al.* 2007a). On the other hand, inducible expression of EGFP-Hec1 abolished the growth of HeLa cells *in vitro* and reduced substantial tumor growth in HeLa xenograft mouse model *in vivo* (Orticello *et al.* 2015). The EGFP-Hec1 expression led to excessive stabilization of kMT attachments, impaired chromosomal segregation and accumulation of multipolar spindles leading to catastrophic mitotic arrest with apoptosis, attenuation of cytokinesis and multinucleation as visualized by live cell imaging (Orticello *et al.* 2015). Implications of overexpression and silencing of Ndc80 in the tumorigenesis of pancreatic cancer has recently been illustrated (Meng *et al.* 2015). A recent study conducted high-throughput lethality RNAi screen of 6000 genes in a panel of ovarian cell lines and identified Hec1 as one of the four genes required for the survival of cancer cells (Sethi *et al.* 2012). Disruption of Ndc80 and Nuf2 complex formation using a small molecule inhibitor, INH1, has been shown to reduce the proliferation in breast cancer cells and reduce tumor growth in a xenograft mouse model (Wu *et al.* 2008). The siRNA-mediated knockdown of Ndc80 and Nuf2 has been shown to induce abnormal mitotic exit and apoptosis in colorectal cancer and gastric cancer cell lines (Kaneko *et al.* 2009).

Targeted knockdown of Knl1 (a constituent of KMN network), a member of the cancer/testis gene family that is predominantly expressed in the testis and widely expressed in primary tumors of different origins, induced apoptotic cell death in human cancer cell lines *in vitro* independent of the p53 status and markedly impeded the growth of implanted tumors *in vivo* (Urata *et al.* 2015). Recently, an interesting study demonstrated that the kinetochore genes are rarely overexpressed in isolation but instead their activation in cancer represents the coordinated and broad induction of core kinetochore module and the associated genes involved in cell cycle and DNA replication (Thiru *et al.* 2014).

Recent studies from our group have shown that Cdt1, a DNA replication licensing protein, is required for robust kMT attachments in human cells in addition to its established role in DNA replication origin licensing (Varma *et al.* 2012). Cdt1 contributes to kMT attachment by binding to the conserved loop region of the Ndc80 complex, but the precise nature of how Cdt1 and the loop domain function together is unknown. Perturbation of function of both the Ndc80 complex and Cdt1 interfere

with the spindle assembly checkpoint, which is defective in cancer cells, and hence, promote them to divide uncontrollably (Diaz-Rodriguez *et al.* 2008, Varma *et al.* 2012). Overexpression of Cdt1 has been associated with many human cancers (Arentson *et al.* 2002, Bravou *et al.* 2005, Petropoulou *et al.* 2008). Specifically, Cdt1 has been found to be overexpressed in some cases of non-small-cell lung carcinomas and lymphoblastic lymphomas with an effect synergistic to the loss of p53 function (Karakaidos *et al.* 2004, Seo *et al.* 2005). With regard to endocrine-related cancers, the DNA replication licensing machinery including Cdt1 is emerging as an important therapeutic target to counter prostate cancer (D'Antonio *et al.* 2009, Majid *et al.* 2010, McCann *et al.* 2014, Wang *et al.* 2014). It is presumable that inhibition of replication licensing interferes with multiple pathways that influence cell proliferation including inhibition of DNA replication and cell cycle arrest, induction of DNA damage and genome instability and/or apoptosis, but it remains to be seen if Cdt1's role in cell division and chromosome segregation contributes to therapeutic potential in this case.

Spindle assembly checkpoint (SAC)

The spindle assembly checkpoint (SAC), a core network of proteins (Mad1, Mad2, Bub1, BubR1, Bub3, Mps1) constitute a surveillance mechanism employed by the cells to monitor the bipolar end-on attachment of each kinetochore to the MT before the transition into anaphase can ensue. Accumulation of these SAC components at the unattached kinetochores serves as a 'wait-signal' to delay the chromosome segregation until proper bi-orientation is established for all the kinetochore pairs. Thus, according to the kMT stabilostat model proposed by Bakhoum & Compton, hypostable kMT attachments fail to adequately satisfy the SAC leading to mitotic delay/arrest, while hyperstable attachments cannot undergo appropriate error correction and persist to carry the attachment errors into anaphase leading to chromosomal missegregation and CIN (Bakhoum & Compton 2012). Loss of SAC function has been attributed to CIN and aneuploidy in several cancers (Kops *et al.* 2005, Herman *et al.* 2015). Interestingly, few SAC proteins also have a direct role in kMT attachments independent of their checkpoint functions. BubR1 recruits PP2A phosphatase to the KT to counteract ABK activity, thereby dephosphorylating ABK substrates and promoting stable kMT attachments (Herman *et al.* 2015). Similarly, Bub1 has been implicated in phosphorylation

of histone H2A resulting in localization of ABK at the centromeres (Herman *et al.* 2015). It is thus evident that there is a cross-talk and a close integration between kMT attachment and SAC. Defects in SAC-independent functions of SAC components may also be responsible for tumor formation (Ricke *et al.* 2008). In functional RNAi screens performed on patient-derived glioblastoma multiforme (GBM, a grade IV astrocytoma, an aggressive and common form of brain cancer in adults) stem-like cells (GSCs), many genes were specifically knocked down and some were found to ameliorate GSC expansion. Two of those identified genes were kinetochore-associated SAC proteins, BubR1 and BuGZ implicated in regulating kMT attachments (Herman *et al.* 2015).

Moreover, Bub1 binding to KT is achieved by Mps1 kinase-mediated phosphorylation of MELT repeats on Knl1, a protein within the KMN network. Mps1 levels were upregulated in a variety of tumors of different origins including bladder, anaplastic thyroid, breast, lung, esophagus and prostate (Colombo *et al.* 2010). A study using a low-molecular-weight inhibitor of Mps1 showed that the inhibitor could decrease the proliferation and viability of both tumor and non-cancer cells but induces apoptosis via poly (ADP-ribose) polymerase (PARP) cleavage specifically in the malignant cells (Kwiatkowski *et al.* 2010). Therefore, even though the specificity and effectiveness of using small-molecule inhibitors against the kinases like Mps1 and Bub1 in terms of targeting only tumor cells and sparing healthy cells warrants further investigation, Mps1 inhibitors have been promising in preclinical trials with some entering into the phase I clinical trials (Dorer *et al.* 2005, Hewitt *et al.* 2010, Santaguida *et al.* 2010, Tardif *et al.* 2011). Similarly, cycloalkenepyrzazole inhibitors of Bub1 kinase activity have been patented but no evidence of efficacy has been presented till date (Tannous *et al.* 2013, Slee *et al.* 2014).

Several human tumor cells have been shown to harbor mutations in mitotic SAC genes encoding Bub1, BubR1, Mad1, Mad2 and in all the three members of Zw10-Rod-Zwilch complex (Kops *et al.* 2005). In prostate and pancreatic cancers, amino acids for Mad1 (R59C, R556C, R359Q and frameshift generating a stop codon at amino acid 318) and Bub1 (Y259C, H265N) are disrupted, respectively (Kops *et al.* 2005). Besides mutations in the SAC proteins, severe repression of Mad2 or BubR1 has been shown to result in massive chromosomal missegregation and apoptosis (Kops *et al.* 2004, Kienitz *et al.* 2005, Janssen & Medema 2013). Prolonged activation of SAC (by generating unattached KTs) or paradoxically opposite

mechanism of inhibiting the SAC (by reducing the levels of Mad2 or BubR1) both have been effective in causing lethality of cancer cells (Kops *et al.* 2004).

Microtubule associated proteins (MAPs)

The intracellular dynamic behavior of MTs is regulated by a concerted balance of MT-stabilizing (MAPs and Tau) and de-stabilizing (stathmin family) proteins, all of them harbor tubulin-binding domain(s) (Bhat & Setaluri 2007). Tau, an extensively investigated MAP is shown to be variably expressed in breast cancer cells and consistent with its MT-stabilizing function, loss of Tau expression was shown to sensitize breast cancer cells to the effect of paclitaxel (Smoter *et al.* 2011). MAP2 (found mainly in neurons) expression is associated with MT stabilization in metastatic melanoma cells leading to cell cycle arrest in G2-M and growth inhibition both *in vivo* and *vitro* (Fang *et al.* 2001). Besides this, expression of MAP2 has been associated with increased sensitivity to MTAs in docetaxel-sensitive pancreatic ductal adenocarcinoma (Veitia *et al.* 2000). Phosphorylated MAP4, a ubiquitously expressed MAP, has been correlated with a decrease in taxol sensitivity in ovarian cancer cells lines (Poruchynsky *et al.* 2001). On the contrary, non-phosphorylated forms of MAP4 are increased in vinblastine-resistant human leukemia cells indicating that the effects of these proteins are cell-type dependent and are more complex than anticipated. High levels of stathmin are reported in a variety of human malignancies (Mistry & Atweh 2002, Mistry *et al.* 2005). Targeting stathmin using antisense in K562 leukemia cell line resulted in abrogation of malignant phenotype (Jeha *et al.* 1996). Similarly, adenovirus-mediated delivery of anti-stathmin ribozymes in prostate cancer cells resulted in massive dose-dependent inhibition of proliferation, accumulation of cells in G2-M and apoptosis (Mistry *et al.* 2005). Altered levels of stathmin and its regulatory pathways are also shown to be responsible for resistance of ovarian cancers to MT-targeting drugs such as paclitaxel (Balachandran *et al.* 2003).

Spindle and kinetochore-associated complex subunit 1 (Ska1) is a microtubule-binding protein at the outer kinetochore that is essential for proper chromosome segregation (Hanisch *et al.* 2006, Schmidt *et al.* 2012). A recent report demonstrates that Ska1 is important in the proliferation of oral adenosquamous carcinoma cells (Zhang *et al.* 2013a). Another group showed that overexpression of Ska accelerates cell division in human

breast cells (Wright & Brooks 2013). Li and coworkers used immunohistochemistry and quantitative RT-PCR to demonstrate Ska1 overexpression in human prostatic intraepithelial neoplasia (Li *et al.* 2014). Their studies also showed that the prostate-specific upregulation of Ska1 in a transgenic mouse model resulted in spontaneous tumorigenesis. Along the same lines, another study revealed that Ska1 expression was significantly higher in papillary thyroid carcinoma (PTC), which accounts for 79–94% of thyroid cancers (Dong *et al.* 2015). Ska2 is also shown to be highly expressed in several cancer cell lines and clinical samples including small-cell lung and breast cancer (Rice *et al.* 2008). Thus, Ska silencing by RNAi might be a potential therapy for these cancer cells. The successful use of small-interfering RNA (RNAi) technology to silence critical gene products has generated significant anti-proliferative and/or pro-apoptotic effects in cell-culture systems or in preclinical animal models (Pai *et al.* 2006, Akar *et al.* 2008, Qin *et al.* 2013, Zhang *et al.* 2013b). Long-lasting RNAi-based gene silencing can also be achieved using lentivirus-based expression systems (Park 2007). Nonetheless, significant obstacles such as *in vivo* delivery, partial suppression of target genes, non-specific immune responses and off-target effects, need to be circumvented before this technology can pave its way to the clinics.

An extremely interesting and novel hypothesis proposed by Tang and Toda is that overexpression of Ndc80 would sequester and unfavorably absorb its accomplices like the Ska complex, Cdt1, the Dam1 complex, TACC-TOG (transforming acidic coiled coil, tumor overexpressed gene), which bind to Ndc80 internal loop region, thereby altering the dynamic flux/equilibrium of these important kinetochore- or spindle-associated proteins in cells (Tang & Toda 2015). Indeed, they demonstrated that overproduction of Ndc80 led to sequestration of its interacting partner, Dis1 in fission yeast, leading to the disruption of the MT structure. Albeit the study did not examine the localization of other loop-interacting proteins, it strongly presents a proof of concept that to compensate for the loss of function of these proteins in response to Ndc80 overexpression, their expression levels can be upregulated. In fact, several cancers report upregulation of Ndc80-loop-interacting proteins like Ska (Sun *et al.* 2014), TACC (Still *et al.* 1999), ch-TOG (colonic and hepatic tumor overexpressed gene) (Charrasse *et al.* 1995), kinesin 8 (Zhang *et al.* 2010) and Cdt1 (Arentson *et al.* 2002, Bravou *et al.* 2005, Petropoulou *et al.* 2008).

Since in each cancer type, there exists a high variability and complexity in the expression of MAP-repertoire that either enhances or reduces tumor sensitivity to MTAs; it is imperative to generate a landscape of tumor-specific MAPs along with its regulatory components for a particular type of cancer. In the wake of advancement in proteome analysis, gene profiling/microarrays, mass spectroscopy and screening small-molecule libraries, this strategy should be deemed suitable to develop efficacious combinatorial therapies (adjunct with MTAs) to alter the expression of a relevant MAP in desired direction or to modulate MAP-tubulin interaction. Targeting the proteins/kinases that regulate the association of MAPs to MTs is another open area of research for the development of future cancer therapeutics.

Mitotic kinases

The process of mitosis utilizes phosphorylation as one of the major regulatory events for centrosome maturation, recruitment of checkpoint and kinetochore-associated proteins, spindle assembly and chromosomal segregation (Malumbres 2011). Therefore, any abnormality in choreography of these highly coordinated events is catastrophic, leading to tumorigenesis. Molecules/inhibitors that target mitosis-specific kinases and phosphatases are proposed to improve the therapeutic index when used alone or with existing drug regimens by impacting slowly-dividing healthy cells to a remarkably lesser extent than the rapidly proliferating cancer cells. Advances in the field of crystallography, structure-based drug designing and chemical genetics can provide powerful platform for such invigorating discoveries. Moreover, dysregulation of mitotic kinases has been associated with uncontrolled and abnormal cell cycle progression; therefore, they can also serve as important diagnostic tools.

The most prominent and exhaustively characterized mitotic kinase is Cdk1 (Cyclin-dependent kinase 1). Based on the cell cycle stage, Cdk1 interacts with its binding partner, Cyclin A (controls entry and progression through G1) or Cyclin B1 (regulates G2-M transition). Cdk1/Cyclin B initiates phosphorylation of its targets allowing entry into mitosis. Degradation of Cyclin B marks the exit from mitosis. Although, mutations or de-regulation of Cdk1 has not been reported in cancer, inhibition of Cdk1 has been shown to induce cell cycle arrest and apoptosis in breast cancer and gastric carcinoma (Lin *et al.* 2006, Li *et al.* 2007b, Schmit & Ahmad 2007, Choi & Kim 2008). An interphase Cdk, Cdk5 has been shown to be

responsible for controlling cell motility and metastasis in prostate cancer (Strock *et al.* 2006). Many small-molecule pharmacological inhibitors targeting Cdks are in different phases of clinical trials with moderate success stories (Asghar *et al.* 2015).

Besides Cdks, Aurora kinase A and B constitute major mitotic kinases. Aurora-A kinase (AAK) is implicated in centrosome separation and spindle formation at the onset of mitosis (Malumbres 2011). RNAi-mediated inhibition of AAK led to a delay in mitotic entry in human cells and conversely, overexpression of AAK led to mitotic irregularities, inhibition of cytokinesis and aneuploidy (Keen & Taylor 2004). AAK overexpression is found in many cancer cell lines like breast, cervical, colorectal, gastric, ovarian, pancreatic and prostate (Sen *et al.* 1997, Carmena & Earnshaw 2003). As discussed in the previous section, ABK contributes to generation of error-free KMT attachments. Similar to AAK, heightened expression of ABK is also associated with colorectal, prostate and thyroid carcinomas (Tatsuka *et al.* 1998, Sorrentino *et al.* 2005, Chieffi *et al.* 2006). Keen and Taylor systematically analyzed the expression levels of *aurora-A*, *aurora-B* and *aurora-C* mRNA in multiple primary tumor samples of different cancers (including breast, lung, colon, prostate, pancreas, liver, skin, stomach, rectum, esophagus, endometrium, cervix, bladder, ovary and thyroid) from diverse stages and origins using microarray analysis (Keen & Taylor 2004). The results demonstrated that both Aurora-A/B were significantly overexpressed in tandem but Aurora-C was neither overexpressed nor correlated with Aurora-A or B expression. The use of small-molecule inhibitors targeting Aurora kinases for cancer therapeutics has been diligently summarized elsewhere (Keen & Taylor 2004, Carvajal *et al.* 2006, Schmidt & Bastians 2007, Lapenna & Giordano 2009, Katayama & Sen 2010).

Another important kinase is polo-like kinase 1 (Plk1), the levels of which are detected during G2-M transition and peaks during mitosis (Malumbres 2011). Plk1 is localized at centrosomes in prophase, and then enriches at the KTs and remains there throughout pro- and metaphase (Lu & Yu 2009). Elevated level of Plk1 has been reported in a plethora of cancers including breast, gastric, endometrial, colorectal, ovarian, thyroid, pancreatic and prostate (Lu & Yu 2009). Concomitantly, knockdown of Plk1 using antisense oligonucleotides or RNAi showed reduced cellular proliferation and a corresponding increase in cell death in many cancer cell lines including prostate cancer (Spankuch-Schmitt *et al.* 2002a,b, Nogawa *et al.* 2005, Reagan-Shaw & Ahmad 2005). Plk1 has also been shown to phosphorylate BubR1 at multiple sites, which

is required for stable kMT attachment and chromosome alignment (Elowe *et al.* 2007). DAB2IP (disabled homolog 2-interacting protein) directly interacts with Plk1 and facilitates the mitotic activation of Plk1. Depletion of DAB2IP in PCa prostate cancer cells significantly reduced mitotic BubR1 phosphorylation, attenuated BubR1 recruitment to the KTs during prometaphase, compromised SAC activity and aberrant chromosomal segregation (Yu *et al.* 2016). However, it is noteworthy that other members of the Plk family, Plk2 and Plk4 function as tumor suppressors and thus the use of a Plk1 inhibitor that does not possess precise specificity would be alarming (Sudakin & Yen 2007).

Motor proteins in mitosis

The outer peripheral region of an unattached kinetochore is a hub for motor proteins like CENP-E kinesin, cytoplasmic dynein–dynactin complex and other SAC proteins (Varma & Salmon 2012). Kinesins are MT-based motor proteins that participate in a myriad of cellular functions like transport of vesicles, organelles, chromosomes and protein complexes and MT movement. All kinesins identified till date are characterized by a ~340 amino acid long motor domain called as ‘head’ harboring an ATP-binding pocket and a MT-binding interface. The kinesins containing the motor domain at the N-terminus typically move toward the actively growing plus-end of MTs, while the ones with the motor domain at the C-terminus possess minus-end directed motility. The ones with a central motor domain utilize ATP for MT depolymerization (Cross & McAinsh 2014, Vicente & Wordeman 2015). So far, 45 *kif* genes have been identified in mammals, which are divided into 14 broad families based on their structure (Chandrasekaran *et al.* 2015). One of the most important mitotic kinesins is the Kif11 (also known as Eg5 or kinesin spindle protein, KSP), a kinesin-5 family member involved in bipolar spindle formation in humans (Blangy *et al.* 1995). Kif15 (a Kinesin-12 family member) has been shown to functionally overlap with Kif11 in the separation of the centrosomes and in the formation of a bipolar spindle (Tanenbaum *et al.* 2009). The outward force created by Kif11 and Kif15 is counteracted by KifC1 (a Kinesin-14 motor), a minus-end directed motor, to help maintain the spindle length (Mountain *et al.* 1999). Several kinesin families contribute to the capture and congression of the chromosomes: kinesin-4 (Kif4), kinesin-7 (Kif10), kinesin-8 (Kif18A), kinesin-10 (Kif22), kinesin-13 (Kif2B, Kif2C) and kinesin-14 (KifC1). The molecular motors like

dynein and Kif10 (also known as CENP-E, a kinesin-7 member) are key players in converting the ‘lateral connection’, wherein the kinetochores first contact the microtubule at the lattice rather than at the microtubule tip, into an ‘end-on connection’ at the plus-end tip of the microtubule to establish robust kMT attachments (Schaar *et al.* 1997, Wood *et al.* 1997, Kapoor *et al.* 2006, Cai *et al.* 2009). CENP-E has also been alluded in integrating kMT attachments and SAC by virtue of its ability to directly bind and modulate the function of BubR1 (Yao *et al.* 2000, Mao *et al.* 2003).

Very early studies in *Drosophila* and *Xenopus* had demonstrated that the inactivation of respective Kif11 homologs, KLP61F and Eg5 led to mitotic arrest with the accumulation of monoastrol spindle (Sawin *et al.* 1992, Heck *et al.* 1993). Moreover, selective inhibition of Eg5 by antibody microinjection into human cells resulted in similar phenotype and mitotic arrest (Blangy *et al.* 1995). The fact that little or no Eg5 is detected in adult non-dividing cells but its expression is prominent in proliferating tissues during development, makes it vulnerable to be exploited as an alluring target (Castillo & Justice 2007). Additionally, overexpression of Eg5 has been found in a variety of solid tumors and leukemias (Salmela & Kallio 2013). Almost a decade ago, identification of monastrol, a small-molecule reversible allosteric inhibitor of the kinesin-5 motor protein/Eg5 aroused interest in using these novel lines of inhibitors to target a protein other than tubulin thereby avoiding the toxicity that encumbers the commonly used MTAs. The success of Eg5 inhibitors has been established in a broad range of tumor cell lines both *in vivo* and *in vitro* (DeBonis *et al.* 2004, Salmela & Kallio 2013). A large number of Eg5 inhibitors, their mechanism of action and their efficacy in clinical trials or challenges has been discussed (Huszar *et al.* 2009).

Following the suite is CENP-E, which is also found to be expressed in elevated levels in several tumors (Wood *et al.* 2008), and thus, its inhibitors like GSK923295A have been developed that induced mitotic delay, apoptosis and tumor regression (Wood *et al.* 2010, Chung *et al.* 2012). Another category of drugs like Lonafarnib blocks farnesylation of CENP-E, depleting this protein from metaphase resulting in defective kMT attachments, reduction in tension between sister kinetochores and activation of SAC (Schafer-Hales *et al.* 2007). Lonafarnib either alone or in combination with other drugs has also proven successful (Ashar *et al.* 2000, Schafer-Hales *et al.* 2007, Penna *et al.* 2017).

Although Eg5 and CENP-E are the two most popularly targeted kinesins, it is exciting to note that the other kinesins are still open for the development of cancer therapeutics. Kif14, a kinesin 3 family member, is overexpressed in ovarian, pancreatic, laryngeal, breast, lung and retinoblastoma tumors and HeLa cells deficient in Kif14 failed to undergo cytokinesis generating binucleate cells that apoptosized after subsequent rounds of impaired mitosis (Carleton *et al.* 2006, Abiatari *et al.* 2009, Theriault *et al.* 2012). An RNAi-based screen of 41 human kinesins identified at least 8 motor proteins in addition to Eg5, CENP-E and Kif14, which when knocked down impacted mitosis and subsequent cytokinesis (Zhu *et al.* 2005). Many reviews expansively document the various studies that highlight the amplification of various kinesin genes in cancer (Yu & Feng 2010, Rath & Kozielski 2012, Chandrasekaran *et al.* 2015). MCAK, a Kinesin-13, family member involved in MT depolymerization, has been shown to be upregulated in a genome-wide expression screen of 81 breast tissues (Nishidate *et al.* 2004). Intriguingly, MCAK expression not only has a relationship with progression of malignancy but also with taxane resistance. Depletion of MCAK has been found to increase the sensitivity of paclitaxel-resistant cells to paclitaxel (Ganguly *et al.* 2011).

Although the plus-end-directed MT motor proteins have been actively studied and targeted for the development of anti-cancer therapeutics, the minus-end-directed motor protein dynein has not received similar attention. Dynein's function in inactivating the SAC has been widely recognized (Wojcik *et al.* 2001, Vallee *et al.* 2006) but how its regulation can contribute to cancer progression remains obscure. Dr Mulder's laboratory previously had shown that km23-1, a light chain of dynein (also called DYNLRB1/LC7-1/robl-1/DNLC2A/DYRB1) is defective in 50% of ovarian cancers (Tang *et al.* 2002). Their recent work demonstrated that overexpression of km23-1 in highly aggressive SKOV-3 human ovarian carcinoma cells (HOCCs) inhibited both monolayer proliferation and anchorage-independent growth of the cells causing an arrest in prometaphase/metaphase and also halted the tumorigenicity of the HOCCs in a xenograft model (Pulipati *et al.* 2011).

Other not 'so popular' yet druggable mitotic protein targets

Since mitosis is an extremely tightly controlled process while tumor cells have limited mechanisms to evade the

targeted pharmacological drugs, the pursuit for identifying other druggable targets is worthwhile. Usually mitotic inhibitors rely on the induction of apoptosis by prolonged mitotic arrest attained via activation of the SAC resulting from aberrant spindle dynamics (Manchado *et al.* 2012). However, even in the presence of active SAC, sometimes, residual APC/C activity is observed, and this phenomenon is coined as 'mitotic slippage' (Brito & Rieder 2006). APC/C-Cdc20, the anaphase-promoting complex, is an E3 ubiquitin ligase that triggers mitotic exit by targeting Cyclin B for degradation, thus inhibiting Cdk1 activity. Mitotic slippage occurs via stochastic Cyclin B degradation even in the presence of an active checkpoint (Brito & Rieder 2006). Since the present day anti-mitotic strategies depend on SAC activation, their effectiveness is dramatically hampered by mitotic slippage, a process that occurs at high rate in cancer cells (Manchado *et al.* 2012). Consequently, it is beneficial to target factors downstream of the checkpoint that do not depend on functional SAC to circumvent slippage issues. Targeting APC/C function might therefore serve as a promising approach as its inhibition would prevent background Cyclin B proteolysis and consequently enhance mitotic arrest while averting mitotic slippage. Moreover, deregulated expression and mutations in APC/C subunits or its co-activators (Cdc20 and Cdh1) have been linked with tumorigenesis (Smolders & Teodoro 2011). Accordingly, depletion of Cdc20 was able to elicit metaphase arrest in cell lines and killing of tumor cells in a mouse model (Huang *et al.* 2009, Manchado *et al.* 2010). TAME, a prodrug that reduced the APC-Cdc20 interaction, has been found to be effective in tumor cells (Zeng *et al.* 2010). Thus, inhibiting mitotic exit by targeting APC/C complex provokes a permanent metaphase arrest by preventing Cyclin B1 degradation, thus irreversibly leading to cell death; bolstering the validity of this approach of targeting mitotic exit.

Constitutive centromere-associated network (CCAN) is a complex of at least ~16 proteins that serves as a bridge between CENP-A in active inner centromeric chromatin and the outer kinetochore proteins including the KMN network that binds to MTs (McAinsh & Meraldi 2011). Recently, a study was conducted wherein the gene expression from 12 different types of human cancers, including breast, lung, liver and prostate was analyzed and the CENP genes were found to be deregulated (Zhang *et al.* 2016). Overexpression of CENP-K in ovarian cancer was found to correlate with poor patient survival and predictive prognosis (Lee *et al.* 2015). Enhanced levels of CENP-A was also identified in several human

malignancies, including hepatocellular carcinoma (Li *et al.* 2011), colorectal cancer (Tomonaga *et al.* 2003), lung adenocarcinoma (Wu *et al.* 2012), poor prognostic impact in estrogen receptor-positive breast cancer (McGovern *et al.* 2012) and epithelial ovarian cancer (Qiu *et al.* 2013). Moreover, CENP-E/F/H/J/T has significant positive hits in the Catalogue of Somatic Mutations in Cancer (COSMIC) database for cancer-associated mutations (Bamford *et al.* 2004). However, a direct correlation between the expression levels of these CENP proteins and cancer remains largely obscure. These studies unequivocally indicate that these centromeric proteins even if currently are not targeted for therapeutics can at least participate as diagnostic biomarkers for tumors.

Condensins are a group of proteins that are required for proper chromosome assembly, condensation and segregation during mitosis (Ono *et al.* 2004). A study has revealed that the changes in nuclear shape observed after Condensin II depletion is a unique property of transformed cells and hence represents an attractive target for cancer cells specifically (George *et al.* 2014).

Protein acetylation on lysine residues is a key post-translational modification that influences several cellular processes (Yang & Seto 2008). This dynamic and reversible modification is mediated by a concerted action of histone acetyltransferases (HATs) and histone deacetylases (HDACs). HDACs play a role in epigenetic modification of chromatin structure in response to environmental changes and are found to be elevated in various human cancers (Marks 2010). One of the HDACs, HDAC3, was shown to be localized on the mitotic spindle and its knockdown resulted in impaired kMT attachments, collapsed spindle surrounded by a dome-like configuration of chromosomes and subsequent activation of SAC in HDAC3-depleted cells (Ishii *et al.* 2008). Fairly recently, a study demonstrated that Aurora B kinase is present in an acetylated form in PC3 prostate cancer cells. Since Aurora B kinase is more active in deacetylated state, inhibition of HDAC3, a substrate for this kinase, resulted in reduced kinase activity and significant defects in Aurora B-dependent mitotic processes, including kMT attachment and chromosome congression (Fadri-Moskwick *et al.* 2012). HDAC inhibitors are recognized as anti-proliferative agents and are thus attractive candidates for anti-cancer therapeutic intervention (Eot-Houllier *et al.* 2009). These drugs arrest the cell cycle at G₁ and G₂/M phase and induce apoptosis, but their precise mechanism of action still remains elusive (Bolden *et al.* 2006).

Advancement in genome-scale loss-of-function screens using RNAi led to the identification of a panorama of essential proteins involved in cell cycle regulation and progression (Mukherji *et al.* 2006). However, most of these are yet to be tested for their potential in arresting tumor growth. One such protein identified in this screen is Haspin, a kinase that is essential for maintaining cohesion between the sister chromatids (Dai & Higgins 2005). The inhibition of this kinase resulted in SAC activation and mitotic arrest reinforcing it as a future plausible cancer target (Petretti *et al.* 2006).

Future challenges on the road from bench to bedside

Despite the convincing preclinical results yielded by new-generation anti-mitotic agents, their clinical efficacy has been promiscuous compared to the microtubule targeting poisons (Manchado *et al.* 2012, Mc Gee 2015). One of the several reasons that afflict development of anti-mitotic therapeutics emanate from the fact that the cells in culture or xenograft animal models have considerably shorter doubling time compared to the actual tumors in the patients (Komlodi-Pasztor *et al.* 2012, Mitchison 2012). This indicates that at a particular time, only a small fraction of tumor cells are undergoing mitosis thus leaving the dormant tumor cells (which are in G₁- or S-phase) completely indifferent or refractory to the drugs that target cell proliferation or aimed at proteins whose expression is highly restricted to only one phase of the cell cycle (like mitotic proteins). This observation also underscores the necessity for frequent drug administration to avoid the likelihood of repopulation of the tumor cells upon clearance of the drug from the system. In addition to the low mitotic index observed in human tumors (estimated to be <1%), the proliferation rate extensively varies in different patients, origins and locations of the tumor (Amadori *et al.* 1997). Furthermore, although these approaches target proteins that are upregulated in cancer cells, thereby providing a narrow/steep therapeutic window to preferentially kill the cancer cells, they still suffer from non-specificity and are thus accompanied by side effects. Another issue underlying the inadequacy of anti-mitotic drugs has been outlined by Mitchison as 'poor drug retention' (Mitchison 2012). For instance, while paclitaxel is retained in the tumor cells for a week and can exert its cytotoxicity for a longer duration, the newer mitosis-selective inhibitors have a median half-life of only ~13 h (Chung *et al.* 2012). Drug resistance has also

been reported for the newer mitosis-selective agents like the Eg5 inhibitor (Tcherniuk *et al.* 2010). There are several ways envisaged to evade these issues: (i) Identifying cancer-specific targets that have oncogenic role outside of mitosis (i.e., important throughout the cell cycle). One such rare candidate is survivin whose expression is cell cycle regulated that escalates to a maximum level during the G2/M phase, consistent with its role in mitosis. Moreover, survivin is overexpressed in practically every human tumor examined (Chan *et al.* 2012). Attenuation of survivin culminated in caspase-dependent apoptosis both *in vitro* and *in vivo* (Chan *et al.* 2010). Downregulation of survivin resulted in severe defects in spindle assembly, compromised SAC, aberrant chromosome motility and incomplete cytokinesis leading to ploidy (Li *et al.* 1999). Thus, targeting survivin is anticipated to transcend the failings of present day anti-mitotics. Indeed, an early-phase trial of survivin inhibitors on humans showed tolerable toxicity and encouraging clinical efficacy (Church & Talbot 2012). Another such molecule is Cdt1 that has been identified very recently to have a mitotic role (specifically in maintaining robust KMT attachments via its interaction with the Hec1 loop) independent of its prototypic role in DNA replication during the G1 phase (Rialland *et al.* 2002, Varma *et al.* 2012). Using high-resolution microscopy, antibody microinjection experiments and biochemistry, it was shown that Cdt1, which gets degraded post S-phase, re-accumulates in G2-M and its inhibition led to a mitotic arrest (Varma *et al.* 2012). Therefore, targeting Cdt1 seems to be an extremely optimistic approach for targeting both types of cancer cells that are undergoing mitosis and also those which are dormant in the G1 phase. Moreover, cancer cells that make aberrant amounts of Cdt1 are expected to experience problems in both replication and mitosis and thus artificially ramping up Cdt1 in such cells is envisioned to push them through an apoptotic pathway. Our laboratory is currently focusing to delineate the precise role of Cdt1 in mitosis, (ii) Mc Gee eloquently proposes that if we re-focus our attention on understanding the post-mitotic signals that integrate with the cell death and senescence pathways, we will discover novel strategies to drive cells down into a precisely defined anti-proliferative route, which is likely to recapitulate and synergize with the current success of anti-mitotic drugs to kill cancer cells before they adapt or develop drug resistance (Mc Gee 2015).

Considering cell cycle deregulation, unscheduled proliferation, genomic instability and chromosomal instability as few of the common features of almost every

type of tumor cells, the recent accelerated progress in the identification and characterization of new cell cycle-specific druggable biomarkers/mechanisms accompanied by concomitant pharmacological advances that will enable us to design and target these wonder drugs better, their lies ahead an exciting era for the development of an effective cancer therapy in the near future.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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