Targeting the Kinesin Spindle Protein: Basic Principles and Clinical Implications

Vasiliki Sarli and Athanassios Giannis

Abstract
Kinesin spindle protein (KSP), a member of the kinesin superfamily of microtubule-based motors, plays a critical role in mitosis as it mediates centrosome separation and bipolar spindle assembly and maintenance. Inhibition of KSP function leads to cell cycle arrest at mitosis with the formation of monoastral microtubule arrays, and ultimately, to cell death. Several KSP inhibitors are currently being studied in clinical trials and provide new opportunities for the development of novel anti-cancer therapeutics alternative from the available microtubule targeting drugs.

Background

The basics of kinesin spindle protein signaling. Several clinically important antimitotics, for example, taxanes, epothilone derivatives, and Vinca alkaloids target tubulin, the basic microtubule subunit. This heterodimeric protein is an important constituent of the cytoskeleton and of the mitotic spindle. Microtubule-directed agents belong to the most successful anticancer agents, and since the introduction of paclitaxel (Taxol) in 1992, these drugs have had a central role in the therapy of breast, lung, ovarian, bladder, and head and neck cancers (1). They also contributed dramatically to a better quality of life of cancer patients and represent milestones in modern chemotherapy (2). However, the use of these agents is associated with serious side effects, such as neurotoxicity, related to the cell cycle or mitotic spindle. A few years ago, Mayer et al. discovered monastrol, the first small molecule able to inhibit the mitotic kinesin Eg5 (kinesin spindle protein, KSP) using a phenotype-based assay and methods of chemical genetics combined with advances in high-throughput screening (3). This discovery may be regarded as a paradigm shift in anticancer drug development. Monastrol inhibits a completely new target, i.e., a protein acting as a cellular motor (mitotic kinesin), expressed during mitosis and involved in cell division. Inhibitors of mitotic kinesins are now being investigated for their therapeutic potential and may represent the next generation of antimitotics.

Kinesins are a large superfamily of motor proteins that participate in various biological phenomena including mitosis and intracellular transport of vesicles and organelles (4). Kinesins consist of a long coiled-coil stalk with a cargo-binding tail at one end and a globular tail domain, usually called the head, at the other (Fig. 1A). The highly conserved motor domain, ~320 residues in size, contains both microtubule and nucleotide binding sides. This domain cooperates with the neck linker ~40 amino acids, to enable motor binding and stepping across the microtubules by converting the chemical energy of ATP hydrolysis to a mechanical force. To date, there are at least 12 kinesins involved in mitosis and meiosis which are responsible for spindle and chromosomal movement. Among them, KSP (HsEg5) is a slow, plus end-directed motor of the kinesin-5 subfamily (5–8). KSP forms a homotetrameric structure capable of binding anti-parallel microtubules and sliding them apart (Fig. 1B and C; ref. 9). It acts during the early stages of mitosis and is responsible for centrosome separation and bipolar spindle assembly, which are essential for proper segregation of chromosomes (10). Failure of KSP function, by immunodepletion or knockdown of KSP mRNA by small interfering RNA, leads to cell cycle arrest in mitosis with monoastral microtubule arrays (11, 12). The important role of KSP in mitotic progression makes it an ideal candidate for drug discovery. Furthermore, it is most abundant in proliferating human tissues and is highly expressed in tumors of the breast, colon, lung, ovary, and uterus.

Targeting KSP provides a novel route for the manipulation of the cell cycle, alternative to antimitotic agents that target tubulin. Tubulin, a heterodimeric protein, is an important constituent of the cytoskeleton and of the mitotic spindle. Microtubule-directed agents belong to the most successful anticancer agents, and since the introduction of paclitaxel (Taxol) in 1992, these drugs have had a central role in the therapy of breast, lung, ovarian, bladder, and head and neck cancers (13). They also contributed dramatically to a better quality of life of cancer patients and represent milestones in modern chemotherapy (2). Agents which target KSP selectively would be expected to act only on cells undergoing cell division, thus making KSP inhibitors mitosis-specific drugs, likely to have fewer side effects than drugs inhibiting other essential microtubule-based processes.}

KSP inhibitors and mechanism of action. A few years ago, Mayer et al. discovered monastrol (1), the first small molecule able to inhibit the mitotic kinesin Eg5 (KSP) using a phenotype-based assay and methods of chemical genetics combined with advances in high-throughput screening (14).
Monastrol is an allosteric inhibitor that binds to an induced-fit pocket 12 Å away from the catalytic center by helices α2 and α3 and loop L5 (15). It binds to KSP and causes both local and distal conformational changes that allow ATP binding, but prevent ADP release. The weak inhibitory activity of monastrol and its non–drug-like properties led to the synthesis of a series of second-generation derivatives with improved cellular potency and solubility (16, 17). Moreover, several other inhibitors that exhibit great chemical diversity have now been reported including adociasulfate-2, terpendole E, dihydropyrazoles, including adociasulfate-2, also acts in a unique manner, as it is not competitive with ATP binding, but interferes with the microtubule-binding site (30). The distinct mechanism of action that KSP inhibitors display could prove especially useful in the clinic for avoiding KSP mutation-mediated resistance.

Mechanisms of cell death induced by KSP inhibitors. Although it is known that KSP inhibitors induce apoptosis after prolonged mitotic arrest, the underlying mechanism is not completely understood. Leizerman et al. have shown that monastrol causes mitotic arrest and induces early apoptosis through mitochondrial membrane depolarization, caspase-8 and caspase-3 activation, and cleavage of poly-ADP-ribose polymerase 1 with different sensitivity in human AGS and HT29 cell lines from gastric and colon carcinoma (31). Similar to other antimitotics that target tubulin, KSP inhibitors activate the spindle assembly checkpoint. The spindle checkpoint ensures the correct segregation of chromosomes by inhibition of cell cycle progression until all chromosome kinetochores are properly attached to the bipolar spindle and chromosomes are aligned at the metaphase plate (32). Well-characterized components of the spindle checkpoint include Mad1, Mad2, Mad3 (BubR1), Bub1, Bub3, and Mps1. The metaphase-anaphase transition occurs as a result of the Cdc20-dependent activation of the anaphase-promoting complex or cyclosome (APC/C). APC/C is a multisubunit E3 ubiquitin ligase that triggers the ubiquitination of a number of mitotic substrates targeting them for destruction by the proteasome.

One hypothesis is that, in response to KSP inhibitors, cells eventually die as a result of overriding the checkpoint. Cells that exit mitosis with unaligned chromosomes will become aneuploid and die. Tao et al. (33) reported that induction of apoptosis in cells treated with a KSP inhibitor happens after long-term mitotic arrest, followed by adaptation and slippage into the next G1 phase. In their studies, KSP-IA (8), a dihydroxyproline small molecule arrests cells in mitosis and induces apoptosis by caspase-dependent death. Cells refractory to slippage or cells with a weakened mitotic checkpoint showed a diminished apoptotic response. It seems that activated checkpoint components such as BubR1 (a protein kinase that is active at unattached kinetochores and contributes to the signal that stops the progression of anaphase), may be required for the induction of apoptosis by KSP-targeted drugs following exit from mitosis. Moreover, KSP-IA was able to induce apoptotic cell death in a p53-independent manner, suggesting that KSP inhibitors could be proved active in p53-deficient tumors.

On the other hand, Chin et al. showed that induction of apoptosis by monastrol is independent of the spindle checkpoint and inhibition of KSP leads to caspase activation and apoptosis in the absence of critical checkpoint proteins such as BubR1 and Mad2 (34). Vijapurkar et al. further elucidated the cellular responses following monastrol-induced mitotic arrest (35). They suggest that the cellular responses induced by monastrol are correlated with overexpression of
BclXL, the antiapoptotic Bcl-2 family protein. Overexpression of BclXL provides a protective mechanism, and its depletion rescues the apoptotic response to monastrol. Furthermore, activation of the death receptor pathway by treatment with Fas receptor agonists sensitized the cells to monastrol-induced cell death, following exit from mitosis.

In another study (36), it was shown that dimethylenastron (a KSP inhibitor) induced apoptosis and simultaneously up-regulated Hsp70 in human multiple myeloma cells. Dimethylenastron-mediated Hsp70 up-regulation is cytoprotective because blocking Hsp70 induction directly by antisense or small interfering RNA or indirectly by inhibitors of the phosphatidylinositol 3-kinase/Akt pathway dramatically increased KSP inhibitor–induced apoptosis. In addition, FTI277, a specific small-molecule inhibitor of farnesyltransferase, interacted synergistically with dimethylenastron in inducing apoptosis through disrupting the Akt/Hsp70 signaling axis. These findings provided the first evidence for KSP inhibitor activity in hematologic malignancy and identify Hsp70 up-regulation as a critical mechanism responsible for modulating myeloma cell sensitivity to KSP inhibitors. These results suggest that a combination of KSP inhibitors with agents abolishing Hsp70 induction would be useful for myeloma therapy and may be of importance for future combination therapies in other malignancies.

Clinical-Translational Advances

Several KSP kinesin inhibitors are currently under development by pharmaceutical or biotechnology companies for cancer treatment. Ispinesib (SB-715992; ref. 12) was the first kinesin inhibitor to enter clinical trials by Cytokinetics and GlaxoSmithKline (37). At present, it represents the most advanced and best studied KSP inhibitor. Below, we summarize and discuss the results from clinical trials involving this quinazolinone derivative, which is now in phase II clinical trials. Phase I studies of ispinesib in patients with solid tumors have been completed. Generally, ispinesib was well-tolerated with an acceptable safety profile with no indications of neurotoxicity. The most common adverse effects were neutropenia, fatigue, anemia, leukopenia, thrombocytopenia, diarrhea, nausea, and vomiting (38–40). The most promising results have been observed in patients with locally advanced or metastatic breast cancer (41). In this study, the predetermined response criteria to progress from stage 1 to stage 2 of the clinical trial were achieved, and patients are currently being...
enrolled in stage 2, in which an additional 25 patients are planned to be enrolled and evaluated. Furthermore, preliminary results from ongoing trials in patients with breast cancer show that ispinesib, in conjunction with capecitabine, indicate an acceptable tolerability profile, and that 8 of 16 patients experienced stable disease for 2 to 6.5 months (42).

Recently, results from the phase II trial of ispinesib in 15 patients with metastatic hepatocellular carcinoma were published (43). Ispinesib was administered as a 1-hour i.v. infusion at a dose of 18 mg/m² once every 3 weeks. In this study, no conclusive evidence of benefit was seen with ispinesib monotherapy. Similar results were obtained from the phase II study in patients with metastatic or recurrent malignant melanoma (44). Although KSP expression seems to be common in melanoma and KSP would be an appropriate target for inhibition, no significant responses were observed in response to ispinesib and further development in malignant melanoma is not recommended. Other preliminary reports of ispinesib in recurrent or metastatic squamous cell carcinoma of the head and neck (45), colorectal cancer, ovarian cancer, and renal cell carcinoma have not indicated significant response rates (46, 47).

Taken together, the results from the first clinical trials of ispinesib are rather disappointing. Nevertheless, it should be taken into account that in these trials, ispinesib was used as monotherapy and further trials are necessary for the evaluation of safety, tolerability, and efficacy of ispinesib in combination with other anticancer therapeutics. For example, cisplatin, a DNA-damaging agent, has been shown to enhance the activity of ispinesib against murine P388 lymphocytic leukemia. In general, it was shown that ispinesib in combination with these standard chemotherapeutic agents has an acceptable tolerability profile with neutropenia as dose-limiting toxicity (48).

Second-generation KSP inhibitors are now in the clinic. SB-743921, a derivative of ispinesib, is 5-fold more potent against KSP ATPase activity and is currently being evaluated in a phase I/II clinical trial in non-Hodgkin’s lymphoma. MK-0731 (Merck) progressed in clinical development has shown antiproliferative activity in many tumor cell lines and significant efficacy in several murine tumor models. In addition, ARRY-520 (Array BioPharma) is currently in a phase I trial in advanced cancer patients and has shown remarkable efficacy in preclinical models of human solid tumors and human leukemias.

The next challenge for the development of KSP inhibitors in the clinic will be multidrug resistance. Resistance to chemotherapy drugs is a principal problem in the treatment of cancer that limits the effectiveness of cytostatic drugs, through the expression of efflux pumps, such as P-glycoprotein (49, 50). It is known that ispinesib resistance, observed in some clinical trials, may be due to multidrug resistance. In fact, preclinical studies indicated that ispinesib may be a substrate for MDR-1, resulting in a variation in response (37). Furthermore, mutations that attenuate ispinesib binding to KSP have already been identified in resistant HCT116 colorectal tumor cells. Two distinct point mutations have been observed resulting in amino acid substitutions within the loop 5 region of the KSP motor domain (51).

Taken together, clinical trials evaluating the efficacy and safety of KSP inhibitors, both as single and in combination with other agents, are currently ongoing. A better understanding of the molecular pathways that KSP inhibitors induce cell death will generate new insights into future therapy. Although ispinesib used as cancer monotherapy proved to be of limited efficacy, its combination with, for example, modulators of the Akt/Hsp70 signaling axis or Fas receptor agonists may be a promising alternative. The cytostatic activity of KSP inhibitors may be augmented by their inhibitory action toward endothelial cell proliferation, resulting in the inhibition of angiogenesis. Furthermore, it should be mentioned that inhibitors of mitotic kinesins may find broad application for the treatment of other proliferative diseases like diabetic retinopathy, pulmonary and liver fibrosis, Sjögren’s syndrome, lupus erythematosus, and lymphoproliferative disorders that develop in patients with a history of autoimmune disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References


Eg5 with antimitotic and antitumor activities. Mol Cancer Ther 2004;3:1079–90.


52. Valentine MT, Fordyce PM, Block SM. Eg5 steps it up! Cell Div 2006;1:31.

Targeting the Kinesin Spindle Protein: Basic Principles and Clinical Implications

Vasiliki Sarli and Athanassios Giannis


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/14/23/7583

Cited articles
This article cites 43 articles, 12 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/14/23/7583.full.html#ref-list-1

Citing articles
This article has been cited by 11 HighWire-hosted articles. Access the articles at:
/content/14/23/7583.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.