

## Role of Stathmin in the Regulation of the Mitotic Spindle: Potential Applications in Cancer Therapy

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### Abstract

Stathmin is a member of a novel class of microtubule-destabilizing proteins that regulate the dynamics of microtubule polymerization and depolymerization. Stathmin promotes microtubule depolymerization during interphase and late mitosis. This microtubule depolymerizing activity of stathmin is regulated by changes in its level of phosphorylation that occur during cell cycle progression. These modifications allow it to play a critical role in the regulation of the dynamic equilibrium of microtubules during different phases of the cell cycle. Stathmin is expressed at high levels in a wide variety of human cancers. Inhibition of stathmin expression in malignant cells interferes with their orderly progression through the cell cycle and abrogates their transformed phenotype. Thus, stathmin provides an attractive molecular target for disrupting the mitotic apparatus and arresting the growth of malignant cells. In this review, we describe the current understanding of the role of stathmin in the regulation of the mitotic spindle and discuss its potential as a therapeutic target of cancer therapy.

**Key Words:** Stathmin, mitotic spindle, microtubule depolymerization, cancer therapy.

WHEN A CELL DIVIDES into two daughter cells, the chromosomes must segregate prior to cell division. This process of chromosome segregation is mediated by a complex structure known as the "mitotic spindle," which is composed primarily of microtubule polymers consisting of  $\alpha$ / $\beta$  tubulin heterodimers. The movement of chromosomes on the mitotic spindle is dependent on the dynamic instability of microtubules, a characteristic property of microtubules that allows them to switch abruptly between states of elongation and rapid shortening (1). The transition from a state of growth to a state of shrinkage is called "catastrophe" and the transition from a state of shrinkage to a state of growth is called "rescue" (1). The dynamics of microtubule polymerization and depolymerization during the cell cycle are regulated by a balance between the activities of two

major classes of proteins, the microtubule-stabilizing and -destabilizing proteins (1, 2). The former class is exemplified by the classic superfamily of microtubule-associated proteins (MAPs), which stabilize the assembled microtubules by suppressing catastrophe (2). The latter class is exemplified by stathmin (3), the KinI family of kinesin-related-proteins (XKCM1 & XKIF2) (4) and the microtubule-severing proteins (katanin, p56 and EF1 $\alpha$ ) (5, 6). All these proteins can destabilize the assembled microtubules by increasing the catastrophe rate of the polymers. These two classes of cellular polymerizers and depolymerizers of microtubules play a critical role in the control of cell division. Recent advances in the molecular understanding of the role of these proteins in cell cycle progression and mitotic spindle assembly provide new opportunities for designing novel therapeutic strategies. For example, perturbation of expression of such microtubule-regulatory proteins might be used to disrupt the mitotic apparatus and arrest the growth of malignant cells. A number of currently available chemotherapeutic agents such as vincristine, vinblastine and Taxol<sup>®</sup> (paclitaxel) exert their antitumor effects by disrupting the normal regulation of the mitotic spindle. This review focuses on the current understanding of the role of

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stathmin in the regulation of the mitotic spindle, and its potential as a novel target for cancer therapy. We also highlight some recent preclinical findings that may one day be translated into more effective cancer therapy.

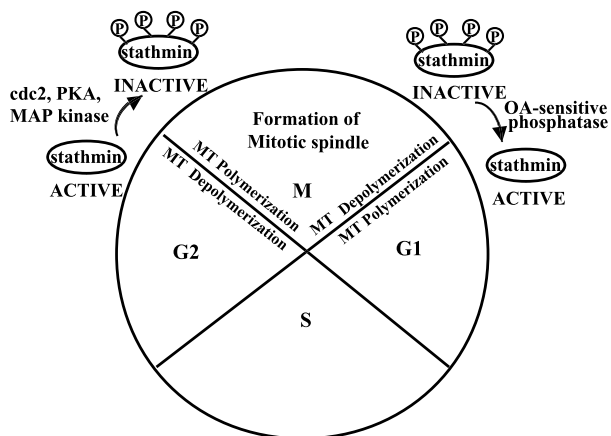
### **Functional Identification of Stathmin as a Microtubule Regulator During Cell Cycle Progression**

Although stathmin was discovered more than a decade ago, its precise function has been elucidated only in the last five years. The initial clue that stathmin may play a role in mitotic progression came from genetic studies that involved either antisense RNA inhibition or forced overproduction of stathmin in leukemic cells. Studies from our own group and others showed that antisense inhibition of stathmin expression results in a marked decrease in the rate of proliferation of K562 erythroleukemic cells and their accumulation in the G2/M phases of the cell cycle (7, 8). Surprisingly, overproduction of stathmin in leukemic cells also resulted in growth inhibition and accumulation of cells in the G2/M phases of the cell cycle (8). These studies suggested that cells have difficulty completing mitosis whenever the level of expression of stathmin is altered. These seemingly paradoxical findings of mitotic arrest with both antisense RNA inhibition and overproduction of stathmin were later explained by a study by Belmont and Mitchison that identified stathmin as a cellular factor that promotes microtubule depolymerization by increasing the rate of catastrophe (3). Thus, it is not surprising that either an increase or decrease in the level of stathmin expression would result in a mitotic arrest. Since the major role of stathmin in mitosis is to promote microtubule depolymerization, inhibition of stathmin should result in increased microtubule polymerization and overexpression of stathmin should result in decreased microtubule polymerization. These predictions were verified experimentally in recent studies by different groups, including our own, by demonstrating increased microtubule polymerization in cells that are deficient in stathmin (9–11) and decreased microtubule content in cells in which stathmin is overexpressed (12).

### **Regulation of the Microtubule Depolymerizing Activity of Stathmin During Cell Cycle Progression**

The microtubule depolymerizing activity of stathmin is regulated by changes in its phospho-

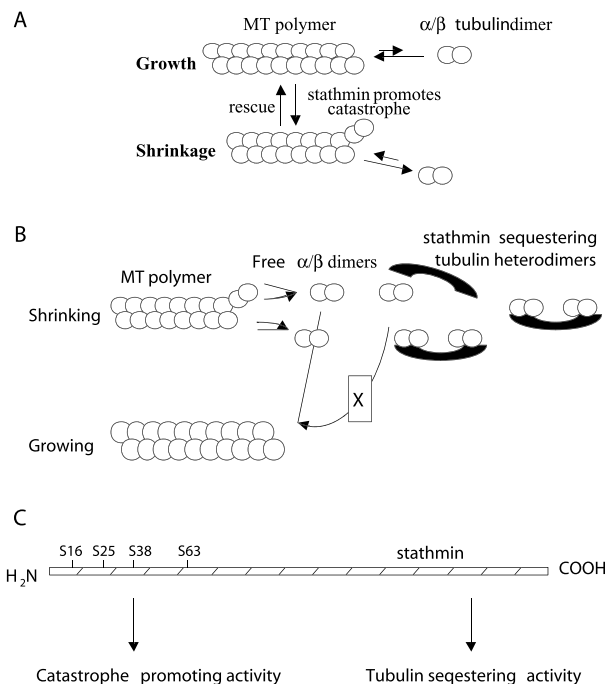
rylation that occur during cell cycle progression (12–14). The level of phosphorylation of stathmin is significantly increased in the mitotic phase of the cell cycle relative to interphase (7). Stathmin is phosphorylated *in vivo* on four distinct serine residues (Ser16, Ser25, Ser38 and Ser63) by different protein kinases, which include cyclin-dependent 2 (cdc2) kinase (7, 15, 16), mitogen-activated protein kinase (17), calmodulin-dependent kinase (18) and cyclic AMP-dependent kinase (16, 19). Phosphorylation of all four serines in mitosis switches off stathmin microtubule-depolymerizing activity and allows the assembly of the mitotic spindle at the onset of mitosis (12). This was demonstrated by Marklund et al., who studied the effects of overexpression of wild-type and cdc2 kinase target site deficient mutants of stathmin on the morphology of the mitotic spindle (12). These studies showed that induced expression of wild-type stathmin results in depolymerization of microtubules in interphase (12). When such cells enter mitosis, stathmin is inactivated by phosphorylation, allowing tubulin to polymerize and assemble the mitotic spindle (12). In contrast, when stathmin mutants that are mutated at kinase target sites are expressed, the cells fail to phosphorylate stathmin, and microtubules can polymerize and form a functional mitotic spindle (12). These observations revealed that inactivation of stathmin by phosphorylation is essential for formation of the mitotic spindle and progression through the cell cycle (12). In a more recent study from our laboratory, dephosphorylation of stathmin by an okadaic acid-sensitive protein phosphatase was found to be necessary for the disassembly of the mitotic spindle, exit from mitosis and entry into a new cycle (10). A general scheme of regulation of polymerization and depolymerization of microtubules during the cell cycle by stathmin phosphorylation is illustrated in Fig. 1. The unphosphorylated stathmin in late interphase promotes depolymerization of microtubules, and phosphorylation of stathmin early in mitosis down-regulates the microtubule depolymerizing activity allowing the mitotic spindle to form. Dephosphorylation of stathmin by an okadaic acid-sensitive protein phosphatase during late mitosis is necessary for spindle disassembly and exit from mitosis. When cells enter a second division cycle, they repolymerize their microtubules in interphase and then depolymerize them again as they enter mitosis. It is not clear yet how the polymerization and depolymerization of microtubules is regulated during interphase.



**Fig. 1.** Regulation of stathmin activity during cell cycle progression. The unphosphorylated form of stathmin in late interphase promotes depolymerization of microtubules, and phosphorylation of stathmin early in mitosis turns off its activity, allowing the mitotic spindle to form. Dephosphorylation of stathmin by an okadaic acid (OA)-sensitive protein phosphatase during late mitosis is necessary for spindle disassembly and exit from mitosis. PKA = protein kinase A; MAP = microtubule-associated protein; cdc2 = cyclin-dependent 2. M, G1, G2, and S are phases of the cell cycle.

### Mechanism(s) of Stathmin-Induced Microtubule Destabilization

At least three major models have been proposed to explain the mechanism(s) by which stathmin destabilizes microtubules (Fig. 2). In the first model, proposed by Belmont and Mitchison, stathmin destabilizes microtubules by increasing the rate of catastrophes (Fig. 2A) (20). A subsequent study by Curmi et al. suggested that stathmin slows elongation but does not act directly on microtubule ends to promote catastrophes (21). The same group also showed that stathmin interacts with two molecules of dimeric  $\alpha/\beta$ -tubulin to form a tight ternary T2S complex (22). This group proposed an alternative model in which stathmin destabilizes microtubules by acting as a tubulin-sequestering protein via the formation of the T2S complex, thus depleting the pool of tubulin available for polymerization (Fig. 2B) (22). These discrepancies were later resolved by Howell et al., who showed that stathmin can have two different functional activities that are compatible with both models under different pH conditions (23). They analyzed truncated stathmin lacking either a C-terminal region or an N-terminal region and showed that different regions of stathmin promote different activities (23). Another study by Larsson et al. demonstrated that stathmin medi-



**Fig. 2.** Proposed models for stathmin-induced destabilization of microtubules. (A) Illustrates the dynamic instability of microtubules. Stathmin promotes depolymerization of microtubules by increasing the rate of catastrophe during the dynamic transitions between phases of growth and shrinkage. (B) Illustrates the tubulin-sequestering activity of stathmin. Stathmin (shown by the curved bars) binds to two molecules of tubulin heterodimer, thereby depleting the pool of tubulin available for polymerization. (C) Illustrates the dual activity of stathmin. The N-terminal region promotes catastrophe-promoting activity and the C-terminal region promotes tubulin-sequestering activity.

ates multiple-region-specific tubulin and microtubule regulatory activities (24). These observations led to a third model, in which stathmin mediates at least two distinct activities: a catastrophe-promoting activity, which requires the N-terminal region of stathmin, and tubulin-sequestering activity that requires the C-terminal region of stathmin (Fig. 2C) (23, 24). More recently, the same group examined the effects of N- and C-terminal truncated mutants of stathmin on interphase microtubules and mitotic spindle microtubules (25). They showed that the catastrophe-promoting activity associated with the N-terminal region of stathmin is sufficient to disrupt the mitotic spindle, while the C-terminal-associated activities are sufficient to destabilize interphase microtubules (25). This suggests that interphase and mitotic microtubules are differentially sensitive to specific activities of stathmin (25). Thus, stathmin appears to function by more than one mechanism. Despite the controversy over the mechanism(s) of activ-

ity of stathmin, there is consensus that stathmin promotes depolymerization of microtubules and is one of the key regulators of cell division.

### **Stathmin Overexpression in Cancer Cells**

Stathmin attracted the attention of many investigators because of its high level of expression in many types of cancer. High levels of expression of stathmin have been observed in a wide variety of human malignancies, including leukemia/lymphoma (26, 27), prostate carcinoma (28), ovarian carcinoma (29), and breast carcinoma (30). The high level of stathmin expression was recently shown to correlate with established prognostic factors in breast carcinoma (31). A previous study from our laboratory showed that antisense inhibition of stathmin expression results in abrogation of the transformed phenotype of leukemic cells *in vitro* and inhibition of tumorigenicity of leukemic cells *in vivo* (32). These findings suggest that high levels of stathmin expression are necessary to maintain the transformed phenotype of leukemic cells.

Although numerous studies have demonstrated high levels of stathmin expression in cancer cells, stathmin does not seem to be directly involved in the process of malignant transformation. This was evident from another study from our laboratory that examined the effects of oncogene-mediated transformation on the level of stathmin expression in an experimental tumor model (33). Our studies showed no direct correlation between the level of stathmin expression and transformation by a wide variety of oncogenes that act through different signaling pathways (33). These findings led us to propose that the high level of expression of stathmin that is frequently observed in tumor cells may be merely a reflection of their increased proliferative activity. This is supported by another study from our laboratory that showed that a high level of stathmin is necessary for maintaining the high proliferative rate of cancer cells (32). However, if the rate of proliferation of the transformed cells is profoundly reduced by inhibiting stathmin expression, they may lose the ability to behave in a malignant fashion, as reflected by their failure to cause tumors in mice (32).

### **Combination of Stathmin Inhibition and Anticancer Agents**

Drugs that inhibit cell division by interfering with microtubule function are widely used

in cancer chemotherapy. Their principal mode of action is exerted either by their ability to bind intracellular tubulin, thereby inhibiting the microtubule assembly and arresting cells in mitosis (e.g., vinca alkaloids), or promoting the assembly of microtubules and stabilizing tubulin polymers by preventing their depolymerization (e.g., Taxol<sup>®</sup>) (34). A number of chemotherapeutic drugs have been examined in combination with antisense strategies that target different proteins whose expression is necessary for the malignant phenotype (35). The antitumor activities of such combinations are additive or synergistic, depending on the cellular targets of the individual therapies. Numerous studies have demonstrated additive effects of combinations in which the cellular targets of the chemotherapeutic agents were different from the targets of the antisense therapies (36, 37). In contrast, the synergistic combination of anti-stathmin therapy and microtubule-directed chemotherapy is particularly attractive, since both therapies target the same microtubule pathway and therefore could theoretically provide an effective form of cancer therapy. In a recent study, we demonstrated that anti-stathmin therapy in combination with non-microtubule-interacting drugs like 5-fluorouracil (5-FU) and doxorubicin results in an additive growth inhibitory effect (38). In contrast, anti-stathmin therapy chemosensitizes K562 leukemic cells to the antitumor effects of Taxol<sup>®</sup> and results in a synergistic growth inhibitory effect *in vitro* (38). The synergistic combination of Taxol<sup>®</sup> and anti-stathmin therapy may provide an effective therapeutic approach that would not be associated with the severe toxicities that result from the use of multiple chemotherapeutic agents with overlapping toxicity profiles.

### **Future Directions**

Given its role in the regulation of mitotic spindle and the dependence of transformed cells on its high level of expression, stathmin provides an attractive molecular target for gene therapy of cancer. Perturbations of expression of proteins like stathmin may disrupt the dynamic equilibrium of microtubule polymerization/depolymerization and induce apoptosis of malignant cells. The discovery of synergistic effects of the combination of Taxol<sup>®</sup> and anti-stathmin therapy *in vitro* is quite intriguing. If these *in vitro* findings are confirmed in an animal model *in vivo*, it may be possible to extrap-

olate this novel therapeutic approach to the treatment of human cancer. However, the success of such an approach depends on the ability to effectively inhibit stathmin expression in the vast majority of cancer cells.

Since stathmin is expressed at very high levels in cancer cells, an anti-stathmin ribozyme-based strategy may be more effective than conventional antisense strategies. Unlike antisense RNA that at best acts stoichiometrically, ribozymes can catalytically cleave multiple target RNA molecules (39, 40). We recently demonstrated the ability of three hammerhead anti-stathmin ribozymes that we designed to cleave synthetic stathmin RNA in a catalytic manner (41). We also showed that these anti-stathmin ribozymes are capable of selective cleavage of native stathmin mRNA when present in a mixture of total RNA isolated from leukemic cells (41). Adenoviral delivery systems aimed at efficient gene transfer of anti-stathmin ribozyme in cancer cells may provide a novel and effective form of cancer gene therapy. Whereas retroviral-mediated gene transfer results in the stable integration of the transgene in the transduced cell, adenovirus gene transfer systems induce an immune response in the host that results in the clearance of transduced cells (42). Although transient expression has obvious limitations for the gene therapy of genetic disease, it may be advantageous for anticancer gene therapy, since elimination of transduced cells is the major aim of such therapy. It may also be possible to combine adenovirus-mediated anti-stathmin ribozyme therapy with pharmacological therapy with Taxol®, to obtain a more potent antiproliferative and antitumor effect. Another avenue of pharmacologic intervention that should be explored is the development of small-molecule inhibitors that block the interaction between stathmin and tubulin. Since stathmin is expressed at very high levels in essentially all types of transformed cells, such therapeutic approaches may find application in the treatment of a wide range of human cancers. The studies that have been conducted so far provide proof of the principle that stathmin could be an excellent target for cancer therapy. More studies are needed before these observations can be translated into effective therapy for human cancer.

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