CANCER GENOMICS & PROTEOMICS 2: 227-238 (2005)

Review

Stathmin in Cell Proliferation and Cancer Progression

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Abstract. *The phosphoprotein stathmin exerts profound influences on cell proliferation, differentiation and in cell motility. These phenotypic features are displayed in response to specific signals imparted to the cell by biological response modifiers. Stathmin functions as a focal point in co-ordinating and directing the cellular signals into specific and defined pathways. Two biological features that characterise cancer are the deregulation of cell proliferation leading to tumour growth and invasive behaviour. Stathmin is up-regulated in many neoplasms and the modulation of its expression correlates with invasion and metastasis and highly proliferating normal tissues. The integrity of the transduction of extracellular signals is essential for the normal functioning of the cellular machinery in cell differentiation, morphogenesis and cell proliferation, apoptosis, growth and senescence. Stathmin mediates these pathways of signalling. Stathmin has been implicated in both G_1-S and G_2-M checkpoint control of cell cycle progression by influencing the dynamics of microtubule formation and progression of the cell cycle. Stathmin appears to exert its regulatory effects at both G_1-S and G_2-M checkpoints by interacting with other cell cycle control proteins such as p53 and rb and with cancer metastasis promoting or inhibiting genes as well as other proteins such as heat shock proteins. Stathmin co-ordinates the signalling by extracellular matrix proteins, and defines intercellular adhesion and cell motility. Therefore, the deregulation of stathmin function would have profound implications in the pathogenesis and progression of cancer.*

Abbreviations: ECM, extracellular matrix; EGF, epidermal growth factor; ER, oestrogen receptors; HCG, human chorionic gonadotropin; HSP, heat shock protein; LH, luteinising hormone; LHRH, luteinising hormone releasing hormone; MAPK, mitogen-activated protein kinase; MT, microtubule; NGF, nerve growth factor; PKC, protein kinase C; PRL, prolactin; TGF, transforming growth factor; TPA, 12-O-tetradecanoylphorbol-13-acetate.

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Key Words: Cancer progression, cell cycle regulation, cell motility, cell proliferation, extracellular matrix, growth factors, heat shock proteins, invasion, metastasis, microtubule dynamics, nm23, prognosis, S100A4, signal transduction, suppressor genes, tumour promoter genes, review.

The phosphoprotein stathmin is a member of a family of phosphoproteins that have long been recognised as playing a major role in cell proliferation, differentiation, development and morphogenesis. It is strongly implicated in the pathogenesis of cancer and other conditions such as Alzheimer's disease and multiple sclerosis. Stathmin participates in cell motility occurring in developing systems as well as in cancer. Stathmin is a ubiquitous cytosolic 19-kDa phosphoprotein. Among other members of note are the neuron-specific RB3 and SCG10, and STMN3 (SCLIP), which show a wide spectrum distribution in human tissues. All the members of the stathmin family are highly conserved phosphoproteins, which are structurally and functionally related (1).

The molecular organisation of stathmin in respect of its two major functions is well established. Stathmin takes part in microtubule (MT) dynamics, with the N-terminal region promoting a rapid transition from elongation to shortening at microtubule ends (a process often described using a somewhat unsavory phrase as catastrophe promotion) occurs in many cellular processes, whereas the C-terminal region inhibits MT polymerisation (2). Stathmin activity can be down-regulated by phosphorylation on its four serines Ser 16, Ser 25, Ser 38 and Ser 63, with all residues requiring to be phosphorylated.

Phosphorylation of the serines suppresses the inhibition of MT polymerisation mediated by the C-terminal region of stathmin. Steinmetz *et al.* (3) have shown that phosphorylation of serine 63 produces transient changes in the molecular

configuration of stathmin and this decimates tubulin binding and suppresses the inhibition of MT polymerisation mediated by the C-terminal region.

Two biological features that characterise cancer are the deregulation of cell proliferation leading to tumour growth and invasive behaviour. Stathmin has been implicated in both these and for this reason its role in cancer progression has been the subject of intensive investigation over the past few years. Stathmin is up-regulated in many neoplasms and the modulation of its expression correlates with invasion and metastasis and highly proliferating normal tissues (4). The stathmin homologue RB3 is one of several genes differentially expressed in non-invasive and invasive cell lines derived from human cancers (5). According to Brattsand *et al.* (6), the expression of stathmin is up-regulated in many leukaemia/lymphoma cell lines. In one cell line, the stathmin content was ten-fold greater than a lymphoblastoid control. Stathmin is over-expressed in malignant ovarian cancers as compared with benign tumours and normal ovarian tissue (7). Tumour cells derived from ovarian borderline tumours and carcinomas have been tested. Cells derived from borderline ovarian tumours show low levels of stathmin when compared with carcinomas (8). This is of great interest in the context of the molecular model proposed for ovarian cancers (9).

The expression of stathmin has been studied in relation to certain established markers of breast cancer malignancy, such as the expression of oestrogen receptors (ER), ploidy, tumour size and grade, together with proliferation markers. Stathmin expression inversely correlates with ER, but positively with other conventional markers, and its over-expression closely reflects the proliferation potential of breast cancers (10, 11). Mistry and Atweh (12) state that over-expression of stathmin is seen in immortalised cells consistent with their higher rates of proliferation, but found no changes in its expression between untransformed and transformed cells, and seem to suggest, therefore, that enhanced stathmin expression is more an attribute of cell proliferation than neoplastic transformation. This contrasts markedly, however, with the finding that prostate epithelial cells transformed by exposure to ionising radiation do show enhanced stathmin expression (13). The involvement of stathmin in the transduction of hormonal and growth factor signals is now well established and, therefore, whether stathmin is related *per se* to cellular transformation is not strictly relevant, indeed totally artificial, in relation to the question of cancer progression since proliferation potential is itself related to the invasive behaviour of cancers. Most recent work on stathmin expression in human cancers seems to suggest a close relationship with progression, which was pointed out some years ago. Interestingly, metastatic disease in lymph nodes and the primary tumour seemed to be similar and, in a small number of human prostate cancers, stathmin expression seemed to be related to patient survival (14).

Mediation of signal transduction by stathmin

The integrity of the transduction of extracellular signals is essential for the normal functioning of the cellular machinery in cell differentiation, morphogenesis and cell proliferation, apoptosis, growth and senescence. The deregulation of the signalling pathways has been shown to bring in its wake major changes in cell proliferation and cellular behaviour that characterise the neoplastic transformation and pathogenesis of cancer. Stathmin and its homologues seem to be implicated in the transduction of signals and functions as a focus for the integration of intracellular signals that influence the proliferative potential and the invasive and metastatic behaviour of cancer engendered by abnormalities associated with the genome.

Stathmin in differentiation and cell proliferation

The expression of stathmin is closely related to the state of cell proliferation and differentiation. It is found to be reduced consonant with progression of differentiation and expression is low in terminally-differentiated cells and, with differentiation being inversely related to proliferation, stathmin expression decreases with decrease in the rate of cell proliferation. The presence of stathmin in an unphosphorylated form was detected in the early stages of erythropoietic differentiation (15).

The involvement of stathmin in growth factor signalling is not a new notion. Hoelscher and Ascoli (16), investigating the effects of EGF (epidermal growth factor) and HCG (human chorionic gonadotropin) in Leydig tumour cells, discovered the induction of a phosphoprotein, which increased steroid production and was able to down-regulate the expression of LH (luteinising hormone)/HCG receptor gene transcription and of adenylyl cyclase. They identified this as stathmin and reported that it is phosphorylated in response to EGF/HCG.

Hummert *et al.* (17) demonstrated this by modulating the differentiation and growth properties of chondrocytes using vitamin D-3 and TGF (transforming growth factor) β1, which promote differentiation and proliferation, respectively, of chondrocytes in culture. Induction of differentiation of PC12 cells by NGF (nerve growth factor) leads to a time-dependent increase of expression of stathmin mRNA. Of much interest is the finding that stathmin mRNA might be localised in the differentiating PC12 cells to the neuronal pole where outgrowths of neurites are formed (18). Thus, stathmin expression is closely linked with neurite differentiation. Pellier-Monnin *et al.* (19) state that stathmin and SCG10 are also up-regulated in olfactory axon regeneration.

The differentiation promoting effects of NGF are negated by stathmin depletion and also stathmin is phosphorylated by the activation of the MAPK (mitogen-activated protein

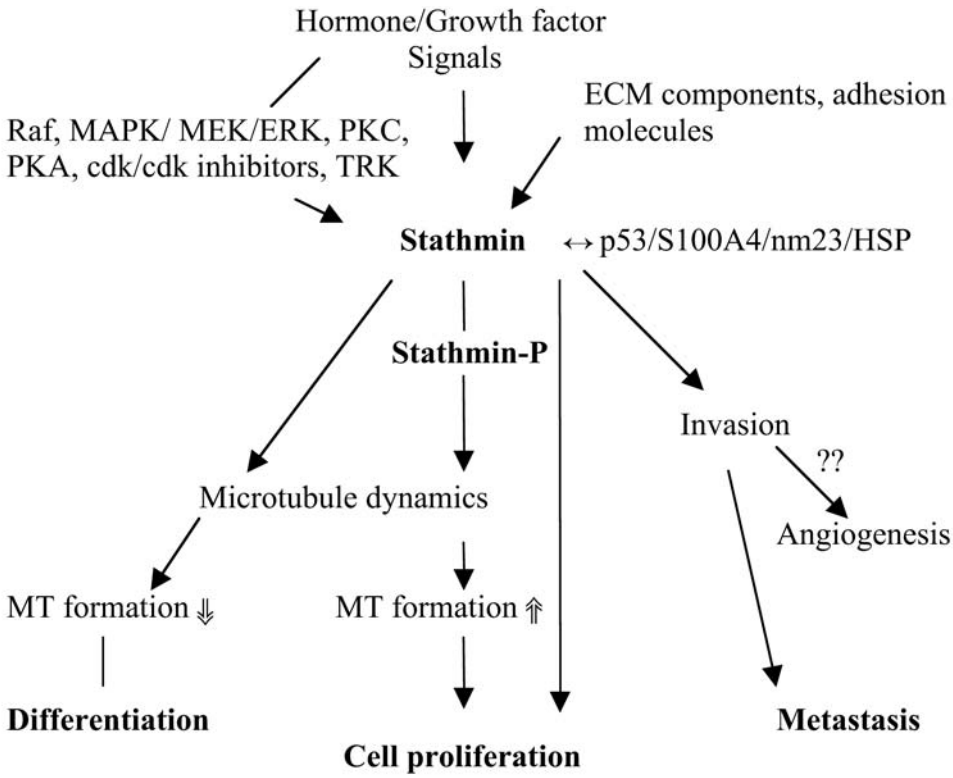

Figure 1. *The stathmin-mediated pathways of signalling in cell differentiation, cell proliferation and cancer invasion and metastasis. The kinases shown all influence and regulate cell differentiation, transformation, and proliferation and apoptosis. MAPK: mitogen activated protein kinase; MEK: MAPK/ERK-kinase; ERK: extracellular signal regulated kinase; tsg: tumour susceptibility gene; HSP: heat shock protein; MT: microtubule; PKC: protein kinase C; TRK: tyrosine receptor kinases. ECM: extracellular matrix*

kinase) pathway by NGF (20). Also the highly-differentiated phaeochromocytomas display a markedly greater stathmin expression than corresponding control adrenal medullary tissue (21). On the other hand, cell proliferation signals imparted by hormones may also be channelled *via* stathmin. For example, LHRH (luteinising hormone releasing hormone)-induced proliferation of αT3-1 gonadotrophe cells is accompanied by the phosphorylation of stathmin. The protein kinase C (PKC) activator 12-O-tetradecanoylphorbol-13-acetate (TPA) also induces stathmin phosphorylation and in both cases phosphorylation is inhibited by the PKC inhibitor staurosporine, which suggests stathmin-mediated transduction of LHRH signal activated by PKC or possibly by some other downstream kinase (22).

Conversely to the situation of the differentiation pathway, it follows that stathmin would be involved in the regulation of cell proliferation. A rapid induction of stathmin expression was shown to accompany liver regeneration (23). This appears to be an indication of stimulation of cell proliferation in response to partial hepatectomy, for changes in stathmin expression relate to the expression of Ki67, a proliferation-related antigen, and are not detectable in resting hepatocytes (24). Exposure of astrocytes to endothelin-1 leads to their transition to the reactive stage. This transition is accompanied by a differential expression or post-translational modifications of several proteins that can be identified with the normal functioning of the cytoskeletal system; notable in the present context, is stathmin and cell adhesion-mediating components such as vinculin, which link ECM components to cytoskeletal structures (25).

Meyer *et al.* (26) demonstrated that PKC-mediated phosphorylation and activation of stathmin occurred upon exposure of Nb2 lymphoma to prolactin (PRL) together with PRL-induced proliferation. As stated earlier, not only does EGF induce the expression of stathmin (16), the growth factor induces stathmin phosphorylation together with proteins displaying the phosphorylation of tyrosine residues (27). The latter, in hindsight, would be compatible with the activation of EGF tyrosine kinase receptors by the ligand. More recently evidence has emerged indicating the involvement of stathmin in cell signalling pathways, leading to proliferation and cytoskeletal organisation, *via* tyrosine kinase. Mirnics *et al.* (28) have reported that in Lyn (a member of the src tyrosine kinase family)-deficient DT40 B cells, stathmin is one the genes that are down-regulated.

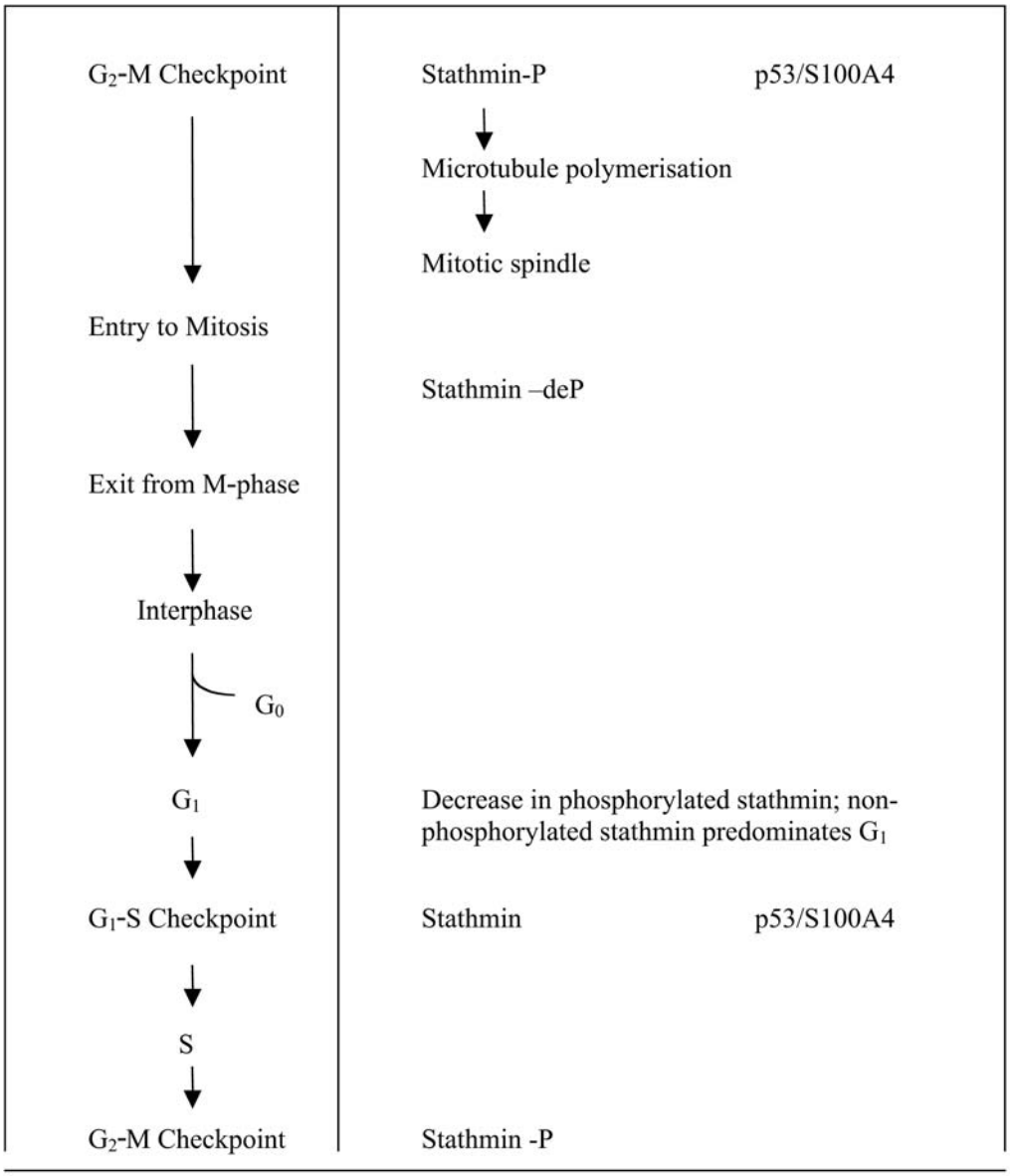

Figure 2. *The involvement of stathmin in G_1-S and G_2-M checkpoint control of cell cycle progression. The phosphorylation status of stathmin changes during the cycle and thus its function. At G_1-S and G_2-M checkpoints, stathmin function might be regulated by interaction with other cell cycle control proteins such as p53 and Rb and with cancer metastasis promoting or inhibiting genes.*

Mode of stathmin function in cell proliferation

There are two distinct signalling pathways in which stathmin appears to be involved in the regulation of cell proliferation. As summarised in Figure 2, stathmin has been implicated in both G_1-S and G_2-M checkpoint control of cell cycle progression. In its unphosphorylated form, stathmin is able to interact with and form a complex [Tubulin2-Stathmin] with soluble tubulin (29). The interaction of stathmin analogues with tubulin occurs by virtue of specific stathmin-like domains (30). This has the effect of sequestering soluble tubulin, leading to the inhibition of microtubule formation. In contrast, upon phosphorylation, the affinity of stathmin for tubulin is reduced and this leads to a promotion of microtubule formation and progression of the cell cycle (Figure 3).

The second pathway by which stathmin could be regulating cell cycle progression is by interacting with the checkpoint control suppressor protein p53. The progression of the cell division cycle is monitored for DNA damage at

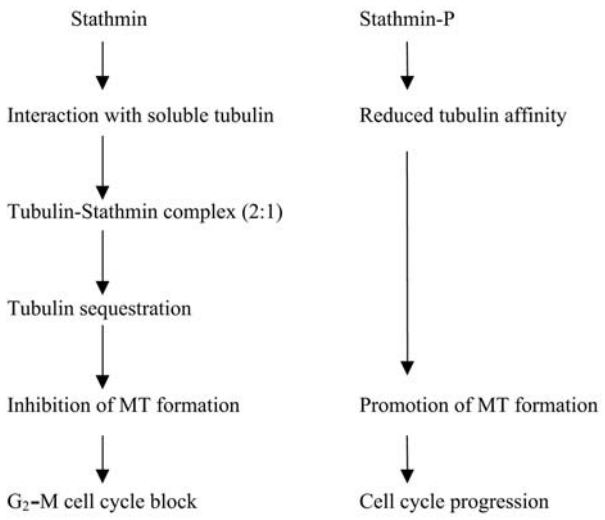

Figure 3. *Stathmin produces G_2-M block by interacting and forming a complex with soluble tubulin, effectively sequestering tubulin and inhibiting the formation of microtubules. Upon phosphorylation, however, the affinity of stathmin for tubulin is reduced and this leads to a promotion of microtubule formation and progression of the cell cycle. Site-specific phosphorylation regulates the activity of stathmin during the transition of cells from interphase to metaphase. Phosphatases might be involved in the dephosphorylation of stathmin at the completion of mitosis and entry of cells into a new cycle. Phosphorylation produces transient changes in the molecular configuration of stathmin and this results in the decimation of tubulin binding and suppresses the inhibition of MT polymersiation mediated by the C-terminal region.*

discrete checkpoints at the G_1-S transition, in the S-phase and at the G_2-M transition. DNA damage is identified and repaired, DNA replication is regulated and the progression of cells through the cell cycle is tightly controlled so that cells with gross genomic abnormalities do not perpetuate by the unrelenting progression of cell division. The repair of damage takes many forms, dependent upon the type of damage sustained, *e.g.* homologous recombination, mismatch repair, maintenance of telomere integrity, chromatin assembly, and the regulation of the assembly of the mitotic spindle and accurate chromosome segregation. The p53 suppressor protein monitors the G_1-S and G_2-M transition together with proteins such as stathmin, detains the cells from transition into S-phase until the damage is repaired or induces the cells into apoptosis if damage is extensive. The S-phase checkpoint controls the rate of replication of DNA and the progression of cells through this phase. The ATR and ATM kinases have been implicated in detecting DNA damage and in the maintenance of telomere length. Whilst ATM is activated in response to double-strand DNA breakage (DSB), DSB in DNA, ATR seems to be required for maintaining the integrity of replication. These kinases in their turn activate checkpoint kinases Chk1 and Chk2 (31, 32). The retinoblastoma susceptibility to Rb protein is another cell cycle regulator that functions at the G_1S checkpoint and p53 functions in conjunction with Rb.

The shared role of cell cycle regulation has led to investigations concerning the co-ordination of p53 signalling *via* stathmin. That stathmin might indeed be so involved is indicated by several lines of evidence. One of these is the direct correlation between the expression of stathmin and p53 expression. Stathmin expression is down-regulated when wild-type p53 is expressed. Not surprisingly, stathmin has also been implicated in the regulatory function of Rb. The latter controls the transcription of genes necessary for the progression of the cell through the cell cycle and appears to achieve this by interacting with E2F transcription factors. Phosphorylation of Rb releases E2F from the Rb-E2F complex and the transcription factor is able to mediate E2F-responsive genes. Furthermore, the Rb-E2F complex is also able to regulate the expression of stathmin and other mitotic regulators in the maintenance of G_2-M checkpoint control (33). The 5' flanking region of the stathmin gene possess four putative binding sites for E2F and, therefore, E2F might mediate the transcription of the stathmin gene (34). Tumour necrosis factor (TNF) seems to be able to induce stathmin phosphorylation. Vancompernolle *et al.* (35) have suggested that TNF-induced apoptosis might be a stathmin-mediated pathway. It might well be that Rb functions in conjunction with stathmin, but equally apoptosis might be brought about by Rb phosphorylation independently of stathmin.

P53 has been shown to repress the transcription of the stathmin gene (36). Johnsen *et al.* (37) have shown that p53 might be inhibiting by repressing stathmin expression. Indeed, p53 appears to be capable of repressing the stathmin promoter. Also, when constitutively over-expressed, stathmin is able to overcome the G_2-M block instigated by p53. Ahn *et al.* (38) had reached similar conclusions with their demonstration that stathmin expression was down-regulated when p53 expression was induced by DNA damage. These findings clearly lend much weight to the argument that stathmin is involved together with p53 in the G_2-M checkpoint control. On the other hand, it is possible that p53 can modulate the ability of stathmin to alter MT dynamics. MT depolymerisation induces the accumulation of p53 leading to cell cycle arrest at G_1-S (39). We know that p53 localises to the microtubules. This might merely indicate its intracellular transport (40). On the other hand, specific localisation of p53 in the mitotic spindle might be crucial in its function (41, 42). The Rb protein might be similarly linked with the structural integrity of microtubules (43). There is little doubt that MT integrity can be closely linked with p53-mediated cell cycle control.

A comparison of the regulation of MT dynamics in breast cancer cell lines has revealed a differential effect of the wild-type and mutated p53 (44). The metastasis promoter protein S100A4 affects MT dynamics, so also does stathmin, which, as we have discussed, can destabilise the cytoskeleton, sequester free tubulin and inhibit tubulin polymerisation. Thus, S100A4 and stathmin share several properties; most prominently, the influence which they exert on cell proliferation, and the postulated interaction with p53. The possibility has also therefore to be considered that S100A4 and p53 might function in collusion to influence the stathmin-mediated inhibition of MT formation. Parker *et al.* (45) found that S100A4 expression parallels that of p53 in murine melanoma cells and suggested further that S100A4 might be interacting with p53 and in this way regulate G_1-S transition. That p53 is one of the targets of S100A4 has been confirmed subsequently (46). Cajone and Sherbet (47) have proposed that S100A4 might be involved in both G_1-S and G_2-M transition by sequestering p53, on basis of the establishment of the down-regulation of stathmin by wild-type 53 and the demonstration that stathmin expression parallel that of S100A4. This pathway, thus, postulates p53 functioning by modulating MT dynamics. It is conceivable that mutated p53 is unable to interact in this way with S100A4 and alter stathmin function. The p53-S100A4 interaction can be viewed in another light, *viz*. as a means by which p53 can counteract S100A4-induced MT depolymerisation.

Heat shock proteins and microtubule dynamics

A family of proteins called heat shock proteins (HSP) has been identified. HSPs are developmentally-regulated proteins and show cell cycle-related expression. HSPs are stress-related proteins, induced by environmental stress imposed by heat shock, viral agents, heavy metals, toxins and oxidants. Some HSPs like HSP70 have been associated with the promotion of cell proliferation, whilst others such as HSP28 have been found to inhibit proliferation. HSP70 seems to interact with p53 and Rb and thus is implicated in cell cycle regulation. Wild-type p53 seems to down-regulate HSP70 promoter whereas, in contrast, mutant p53 transactivates it (48, 49). Furthermore, the p53–/– phenotype shows more marked enhancement of HSP70 than the p53+/+ (50). The small molecular weight HSP, HSP28, possesses growth inhibitory property (51). It has been shown that enhanced expression of HSP28 is associated with marked reductions in the size of the S-phase fraction together with the down-regulation of S100A4 (52, 53). This suggests HSPs might be functioning by interacting with p53/S100A4 in influencing cell cycle progression. Many HSPs are associated with microtubules as, for instance, HSP105a (54). HSP40 binds to the keratins 18 (K8/18) component of intermediate filaments (55). The microtubule binding protein Mip-90 bears marked sequence homology to HSP90 (56). HSP70 seems not only to bind to microtubules, but also to maintain the integrity of microtubules (57). HSPs are known to interact with tubulin dimers (58) and in this way they might affect cell cycle progression. More relevant in terms of cell cycle regulation is the demonstration that the binding of HSPs to tubulin modulates the dynamics of microtubule formation. Garnier *et al.* (59) showed that HSP90 binds *in vitro* to tubulin dimer and inhibits tubulin polymerisation. This would be expected to promote cell cycle progression.

In an alternative pathway, stathmin could be viewed as a downstream effector of this pathway of signalling, which might also explain the differences between the larger HSP70/90s and the smaller HSPs. Stathmin is induced in Jurkat cells exposed to heat shock (60). Although the functional significance is yet to be demonstrated, heat shock and chemical stress do indeed induce phosphorylation of stathmin on serine 25 (61). Serine 25 and serine 38 are cyclin-dependent kinase phosphorylation targets and serines 16 and 63 are phosphorylated by other kinases. The phosphorylation of serines 25 and 38 alone are insufficient, but phosphorylation of serines 16 and 23 seems to depend upon serines 25 and 38 being phosphorylated. Thus, the phosphorylation of all four serines might be required for regulating stathmin function (62). The heat shock cognate protein HSC70 (HSP70 family) does interact with stathmin and its interaction is dependent upon the phosphorylation state of stathmin (63). In contrast, using a mouse model of familial amyotrophic lateral sclerosis, Strey *et al.* (64) have shown that deregulation of stathmin and a decrease of its expression as phosphorylated isoform was associated with an up regulation of expression of HSP25 and 27. It would be expected that reduction of phosphorylation would result in inhibition of microtubule formation and therefore inhibit cell cycle progression (Figures 1 and 3). This would explain the growth inhibitory properties of HSP28 mentioned earlier.

Stathmin in cancer invasion and metastasis

A distinctive phase of cancer progression is the acquisition by cancer cells of the ability to invade host tissue stroma and the invasion and dissemination into the vascular and lymphatic compartment. These faculties are a composite effect of a number of cellular changes, which include unregulated cell proliferation, induction of neovascularisation and remodelling of the ECM, resulting in alterations of intercellular adhesion and ECM-mediated signalling. The latter alters the cytoskeletal dynamics, thus producing not only changes in the physical features of the cells that are conducive to invasive behaviour, but also altering the signalling pathway itself. With cytoskeletal dynamics figuring

so prominently in all these aspects of aberrant cell behaviour, it is hardly surprising that stathmin forms an important component of the pathway of the flow of information.

The remodelling of the ECM is a dynamic process and includes the excision, regeneration and spatial redistribution of ECM components and the expression of enzyme systems that participate in the release of tumour cells and enables them to invade the newly-formed vessels surrounding the tumour. ECM components participate in cell signalling required in cell migration. For example, directional migration of neuronal growth cones is guided by ECM components. For instance, laminin and fibronectin markedly alter growth cone behaviour, which is effected by filopodial contact with the substratum. This suggests that ECM components direct axon outgrowth by intracellular signalling through growth cone filopodia (65).

Another example is provided by the demonstration that retinal growth cone behaviour and cell axonal directional responses can not only be modulated by ECM components but the changes in behaviour result from signalling involving microtubule dynamics co-ordinated by stathmin (66). It would be worth reiterating in the context of cancer progression that stathmin expression and its modulation correlate with invasive and metastasis potential of many human neoplasms. Stathmin is up-regulated in highly proliferating normal tissues (4). Differential expression of a stathmin homologue has been encountered in non-invasive and invasive cell lines derived from human cancers (5).

The invasive behaviour of cancer cells is attributable to the expression or loss of intercellular adhesion molecules. A prime example is the expression of cadherins, which are transmembrane glycoproteins. These are linked with the cytoskeletal structure. The modulation of adhesive and invasive behaviour seems to result from abnormalities associated with cadherin/cytoskeletal linkage. Loss of cadherins and abnormalities in the linking proteins, vinculin and catenin, invariably result in changes in the biological behaviour of cells. Differentiation inducing agents such as retinoic acid also up regulate cadherin expression (51). Indeed for these reasons, cadherins are regarded as tumour suppressors. There is an obvious link-up of cadherins with cytoskeletal structures and the function of this assembly in bringing about changes in phenotypic behaviour. It is of considerable interest therefore that stathmin is involved in a density-dependent manner in cell cultures that typify cell to cell contacts and contact inhibition of proliferation. E-cadherin antibodies have been found to interfere with intercellular contacts and also in parallel down-regulate stathmin expression (67). Stathmin appears to be phosphorylated by Rac1 and p21-activated kinases (68). In the absence of any clear migration assays, this evidence can be seen as circumstantial in nature. Nonetheless, the association of these kinases in the microtubule dynamics at the leading edge of invading cells is another line of evidence linking stathmin activation with invasion.

Stathmin and angiogenesis

The successful metastatic spread of cancer requires cancer cells not only to acquire the ability of local invasion, but also the ability to cross the endothelial barrier into the vascular system. At the present time, there is very little information about the relevance of stathmin in the process of angiogenesis or neovascularisation. Certain angiogenic agents appear to have the ability to induce changes in gene expression and among them is stathmin. Zhou *et al.* (69) found that inhibition of angiotensin-1 receptor (of angiotensin II) down-regulates the expression of stathmin and FGF receptor genes, together with the down-regulation of the tyrosine kinase gene. This seems to happen independently of the normal physiological function of angiotensin and its receptor. Several other growth factors, such as TGF and TFN and steroid hormones oestrogen and progesterone, possess angiogenic properties and also influence the expression of stathmin. However, one should reiterate that, as yet, there is no direct evidence linking stathmin with angiogenesis.

Some findings relating to stathmin expression in cells exposed to stress factors (47) have drawn attention to the possibility of the involvement of stathmin in angiogenesis. TPA and 4-hydroxy-2-nonenal (HNE) are capable of inducing hemeoxygenase (HO)-1. HO-1 possesses regulatory sequences for many transcription factors that are associated with cell proliferation, endothelial cell differentiation and, therefore, it is identifiable with angiogenesis (70). Furthermore, both TPA and HNE modulate stathmin function. Although this does not constitute direct incontrovertible evidence, it might be worthwhile drawing attention to these so that some interest might be generated in the area of stathmin function, which has received very little attention to date.

Miyashita *et al.* (71) have recently shown that VEZF1 (vascular endothelial zinc finger 1) regulates angiogenesis in mouse embryos. Using antisense strategy, they showed that down-regulation of VEZF1 resulted in the inhibition of proliferation, migration and formation of an angiogenic network of endothelial cells, but transfection of VEZF1 cDNA had the opposite effect. The down-regulation of VEZF1 also down-regulated stathmin expression and in VEZF1 cDNA-transfected cells stathmin expression was up-regulated. These findings might implicate stathmin in angiogenesis, albeit in murine embryos.

Therapeutic implication of stathmin function and its deregulation

The extensive involvement of stathmin in signalling mechanisms and the consequences of its deregulation has inevitably suggested the use of stathmin as an aid in cancer treatment and patient management. Several avenues of

approach might be considered. One useful approach would be to determine whether one could glean useful information about disease progression and prognosis, drug resistance from the modulation of stathmin expression and for determining treatment modality.

As discussed previously, stathmin can differentiate between borderline and malignant ovarian tumours. Furthermore, Chen *et al.* (72) found that high stathmin expression was associated with poorly-differentiated lung adenocarcinomas. Therefore, it would be reasonable to expect stathmin levels to relate to tumour progression. Over-expression of stathmin in breast cancer seems to correlate with loss of ER and PgR, which indicates more aggressive disease. Stathmin expression also correlated with tumour grade and proliferative state (11). The question arises as to how stathmin expression relates to prognosis. A preliminary study of prostate cancer patients showed a significant correlation between stathmin positivity and patient survival (14). Neben *et al.* (73) noticed that high expression of stathmin and Aurora kinase STK15, among other markers, was associated with poor overall survival in patients with medulloblastoma. The over-expression of Aurora kinase in association with stathmin is of more than mere passing interest, for Aurora kinases are centrosome-associated serine/threonine kinases, and themselves appear to play an important part in cell division as well as in tumour development (32). However, no functional link has been established between stathmin and the kinase.

Some effort has been expended to determine if stathmin expression can be used as a guide for the use of microtubule-targeting drugs in cancer treatment. The microtubule-binding drug Paclitaxel (Taxol) inhibits depolymerisation and a consequence of this is that the dissolution of the mitotic spindle is inhibited, leading to a block of cell division. In contrast, vinca alkaloids bind to beta-tubulin in the α/β-tubulin heterodimer and disrupt microtubule dynamics, which inhibits the formation of the mitotic spindle. Paclitaxel-resistant ovarian cancer cell lines show over-expression of stathmin (74). Breast cancer cell lines differing in stathmin expression and cell lines forced to over-express stathmin showed decreased sensitivity to paclitaxel and vinblastine. In contrast, sensitivity to drugs that do not affect MT dynamics bore no relationship to stathmin content (75). Human carcinoma cells stably transfected with stathmin cDNA are highly sensitive to vindesine and vincristine (76). Rosell *et al.* (77) have tested the expression of stathmin and β-tubulin III for predicting response to chemotherapy in a randomised trial of non-small cell lung cancer patients. They found time for progression of the disease was related to the expression of both β-tubulin III and stathmin in vinorelbine (vinca alkaloid)/cisplatin and to a lesser extent in the paclitaxel/ carboplatin arm of the trial. Rosell *et al.* (77) have therefore strongly advocated a prospective study.

Another approach would be to check if stathmin might itself be a therapeutic target of cancer therapy. Mistry *et al.* (78) have suggested this approach as an anti-angiogenesis therapy. Antisense strategy offered a useful mode of targeting stathmin in leukaemic cells, thus achieving growth inhibition (79). Mistry *et al.* (80) also demonstrated the potential efficacy of employing the ribozyme strategy for targeting stathmin. Anti-stathmin ribozyme cleaved stathmin RNA with high selectivity and at the rate of several RNA molecules per ribozyme molecule. This approach does offer some promise. However, the efficacy of directly targeting stathmin depends upon the pattern and topography of its distribution, especially with regard to the heterogeneity of distribution in the tumour.

Concluding remarks

Stathmin exerts profound influences on cell proliferation, differentiation and in cell motility. Most of these effects are a phenotypic display of properties generated in response to specific signals imparted to the cell by biological response modifiers. The body of evidence presented here underscores the importance of stathmin as a focal point in co-ordinating and directing the cellular signals into specific and defined pathways. Thus, in the scenario presented here, not only are the opposing features of proliferation and differentiation co-ordinated, but also co-ordinated are the pathways of signalling used by cell cycle regulator genes and metastasis suppressor and promoter genes, together with pathways of signalling adopted by ECM proteins to defining intercellular adhesion and cell motility. Therefore, deregulation of stathmin function would have serious implications for the biological behaviour of the cell that could lead to the pathogenesis and progression of cancer. The use of stathmin in tailoring cancer treatment and as an aid in patient management is a potential area of application that has not received the degree of attention it deserves and the currently available data is best described as meagre. It is hoped that future research will focus attention on this highly constructive and beneficial feature of cancer management.

Acknowledgements

We would like to thank Dr M.S. Lakshmi for reading the manuscript and making helpful suggestions and Professor Oliver Hinton for the provision of excellent research facilities and much encouragement.

References

1 Bieche I, Maucuer A, Laurendeau I, Lachkar S, Spano AJ, Frankfurter A, Levy P, Manceau V, Sobel A, Vidaud M and Curmi PA: Expression of stathmin family genes in human tissues: non-neural-restricted expression for SCLIP. Genomics *81*: 400-410, 2003.

2 Segerman B, Holmfeldt P, Morabito J, Cassimeris L and Gullberg M: Autonomous and phosphorylation-responsive microtubule-regulating activities of the N-terminus of Op18/stathmin. J Cell Sci *116*: 197-205, 2003.
3 Steinmetz MO, Jahnke W, Towbin H, Garcia-Echeverria C, Voshol H, Muller D and van Oostrum J: Phosphorylation disrupts the central helix in Op18/stathmin and suppresses binding to tubulin. EMBO Rep *2*: 505-510, 2001.
4 Baldassarre G, Belletti B, Nicoloso MS, Schiappacassi M, Vecchione A, Spessotto P, Morrione A, Canzonieri V and Colombatti A: p27(Kip1)-stathmin interaction influences sarcoma cell migration and invasion. Cancer Cell *7*: 51-63, 2005.
5 Walter-Yohrling, Cao XH, Callahan M, Weber W, Morgenbesser S, Madden SL, Wang C and Teicher BA: Identification of genes expressed in malignant cells that promote invasion. Cancer Res *63*: 8939-8947, 2003.
6 Brattsand G, Roos G, Marklund U, Ueda H, Landberg G, Nanberg E, Sideras P and Gullberg M: Quantitative analysis of the expression and regulation of an activation regulated phosphoprotein (oncoprotein 18) in normal and neoplastic cells. Leukemia *7*: 569-579, 1993.
7 Price DK, Ball JR, Bahrani-Mostafavi Z, Vachris JC, Kaufman JS, Naumann RW, Higgins RV and Hall JB: The phosphoprotein Op18/stathmin is differentially expressed in ovarian cancer. Cancer Invest *18*: 722-730, 2000.
8 Alaiya AA, Franzen B, Fujioka K, Moberger B, Schedvins K, Silversvard C, Linder S and Auer G: Phenotypic analysis of ovarian carcinoma: polypeptide expression in benign, borderline and malignant tumours. Int J Cancer *73*: 678-683, 1997.
9 Sherbet GV and Patil DB: Molecular progression of ovarian cancer. *In*: Molecular and Cellular Pathology of Cancer Progression and Prognosis, Sherbet GV (ed.). Transworld Research Network, Trivandrum, 2005.
10 Brattsand G: Correlation of oncoprotein 18/stathmin expression in human breast cancer with established prognostic factors. Br J Cancer *83*: 311-318, 2000.
11 Curmi PA, Nogues C, Lachkar S, Carelle N, Gonthier MP, Sobel A, Lidereau R and Bieche I: Overexpression of stathmin in breast carcinomas points to highly proliferative tumours. Br J Cancer *82*: 142-150, 2000.
12 Mistry SJ and Atweh GF: Stathmin expression in immortalized and oncogene transformed cells. Anticancer Res *19*: 573-577, 1999.
13 Prasad SC, Thraves PJ, Soldatenkov VA, Varghese S and Dritschilo A: Differential expression of stathmin during neoplastic conversion of human prostate epithelial cells is reversed by hypomethylating agent, 5-azacytidine. Int J Oncol *14*: 529-534, 1999.
14 Friedrich B, Gronberg H, Landstrom M, Gullberg M and Bergh A: Differentiation-stage specific expression of oncoprotein-18 in human rat prostatic adenocarcinoma. Prostate *27*: 102-109, 1995.
15 Rabilloud T, Berthier R, Valette C, Garin J and Lawrence LJ: Induction of stathmin expression during erythropoietic differentiation. Cell Growth Differ *6*: 1307-1314, 1995.
16 Hoelscher SR and Ascoli M: Leydig tumour-cell protein phosphorylated in response to stimulation with choriogonadotropin or epidermal growth factor is indentified as stathmin. Endocrinology *135*: 662-666, 1994.
17 Hummert TW, Schwartz Z, Sylvia VL, Dean DD and Boyan BD: Stathmin levels in growth plate chondrocytes are modulated by vitamin D-3 metabolites and transforming growth factor-beta 1 and are associated with proliferation. Endocrine *15*: 93-101, 2001.
18 Hannan AJ, Henke RC, Weinberger RP, Sentry JW and Jeffrey PL: Differential induction and intracellular localization of SCG10 messenger RNA is associated with neuronal differentiation. Neuroscience *72*: 889-900, 1996.
19 Pellier-Monnin V, Astic L, Bichet S, Riederer BM and Grenningloh G: Expression of SCG10 and stathmin proteins in the rat olfactory system during development and axonal regeneration. J Comp Neurol *433*: 239-254, 2001.
20 DiPaolo G, Pellier V, Catsicas M, Antonsson B, Catsicas S and Grenningloh G: The phosphoprotein stathmin is essential for nerve growth factor-stimulated differentiation. J Cell Biol *133*: 1383-1390, 1996.
21 Takekoshi K, Nomura F, Isobe K, Motooka M, Nammoku T and Nakai T: Identification and initial characterization of stathmin by the differential display method in nerve growth factor-treated PC12 cells. Eur J Endocrinol *138*: 707-712, 1998.
22 Drouva SV, Poulin B, Manceau V and Sobel A: Luteinizing hormone-releasing hormone-signal transduction and stathmin phosphorylation in the gonadotrope alpha T3-1 cell line. Endocrinology *139*: 2235-2239, 1998.
23 Okazaki T, Himi T, Peterson C and Mori N: Induction of stathmin messenger RNA during liver regeneration. FEBS Lett *336*: 8-12, 1993.
24 Rowlands DC, Harrison RF, Jones NA, Williams A, Hubscher SG and Brown G: Stathmin is expressed by the proliferating hepatocytes during liver regeneration. J Clin Pathol Clin Mol Pathol *48*: M88-M92, 1995.
25 Egnaczyk GF, Pomonis JD, Schmidt JA, Rogers SD, Peters C, Ghilardi JR, Mantyh PW and Maggio JE: Proteomic analysis of the reactive phenotype of astrocytes following endothelin-1 exposure. Proteomics *3*: 689-698, 2003.
26 Meyer N, Prentice DA, Fox MT and Hughes JP: Prolactin-induced proliferation of the Nb2 T-lymphoma is associated with protein kinase C-dependent phosphorylation of stathmin. Endocrinology *131*: 1977-1984, 1992.
27 Ji H. Baldwin GS, Burgess AW, Moritz RL, Ward LD and Simpson RJ: Epidermal growth factor induces serine phosphorylation of stathmin in a human colon carcinoma cell line (LIM-1215). J Biol Chem *268*: 13396-13405, 1993.
28 Mirnics ZK,Caudell E, Gao Y, Kuwahara K, Sakaguchi N, Kurosaki T, Burnside J, Mirnics K and Corey SJ: Microarray analysis of Lyn-deficient B cells reveals germinal center-associated nuclear protein and other genes associated with the lymphoid germinal center. J Immunol *172*: 4133-4141, 2004.
29 Jourdain L, Curmi P, Sobel A, Pantaloni D and Carlier MF: Stathmin: A tubulin-sequestering protein which forms a ternary T2S complex with two tubulin molecules. Biochemistry *36*: 19817-10821, 1997.
30 Charbaut E, Curmi PA, Ozon S, Lachkar S, Redeker V and Sobel A: Stathmin family proteins display specific molecular and tubulin binding properties. J Biol Chem *276*: 16146-16154, 2001.
31 Sherbet GV: Genetic Recombination in Cancer. Academic/Elsevier, London, 2003.
32 Sherbet GV: DNA ploidy and the progression of cancer. *In*: Molecular and Cellular Pathology of Cancer Progression and Prognosis, Sherbet GV (ed.). Transworld Research Network, Trivandrum, 2005.
33 Polager S and Ginsberg D: E2F mediates sustained G(2) arrest and down-regulation of stathmin and AIM-1 expression in response to genotoxic stress. J Biol Chem *278*: 1443-1449, 2003.

34 Polzin RG, Benlhabib H, Trepel J and Herrera JE: E2F sites in the Op18 promoter are required for high level of expression in the human prostate carcinoma cell line PC-3-M. Gene *341*: 209-218, 2004.
35 Vancompernolle K, Boonefaes T, Mann M, Fiers W and Grooten J: Tumor necrosis factor-induced microtubule stabilization mediated by hyperphosphorylated oncoprotein 18 promotes cell death. J Biol Chem *275*: 33876-33882, 2000.
36 Murphy M, Ahn J, Walker KK, Hoffman WH, Evans RM, Levine AJ and George DL: Transcriptional repression by wild-type p53 utilizes histone deacetylases, mediated by interaction with mSin3a. Genes Dev *13*: 2490-250, 1999.
37 Johnsen JI, Aurelio ON, Kwaja Z, Jorgensen GE, Pellegata NS, Plattner R, Stanbridge EJ and Cajot JF: p53-mediated negative regulation of stathmin/Op18 expression is associated with G(2)/M cell-cycle arrest. Int J Cancer *88*: 695-691, 2000.
38 Ahn J, Murphy M, Kratowicz S, Wang A, Levine AJ and George DL: Down-regulation of the stathmin/Op18 and FKB25 genes following p53 induction. Oncogene *18*: 5954-5958, 1999.
39 Sablina AA, Chumakov PM, Levine AJ and Kopnin BP: p53 activation in response to microtubule disruption is mediated by integrin-Erk signaling. Oncogene *20*: 899-909, 2001.
40 Giannakakou P, Sackett DL, Ward Y, Webster KR, Blagosklonny MV and Fojo T: P53 is associated with cellular microtubules and is transported to the nucleus by dynein. Nature Cell Biol *2*: 709-717, 2000.
41 Morris VB, Brammall J, Noble J and Reddel R: P53 localizes to the centrosomes and spindles of mitotic cells in the embryonic chick epiblast, human cell lines, and a human primary culture: an immunofluorescence study. Exp Cell Res *256*: 122-130, 2000.
42 Ciciarello M, Mangiacasale R, Casenghi M, Limongi MZ, D'Angelo M, Soddu S, Lavia P and Cundari E: p53 displacement from centrosomes and p53-mediated G(1) arrest following transient inhibition of the mitotic spindle. J Biol Chem *276*: 19205-19213, 2001.
43 Khan SH and Wahl GM: p53 and pRb prevent rereplication in response to microtubule inhibitors by mediating a reversible G(1) arrest. Cancer Res *58*: 396-401, 1998.
44 Galmarini CM, Kamath K, Vanier-Viornery A, Hervieu V, Peiller E, Falette N, Puisieux A, Jordan MA and Dumontet C: Drug resistance associated with loss of p53 involves extensive alterations in microtubule composition and dynamics. Br J Cancer *88*: 1793-1799, 2003.
45 Parker C, Lakshmi MS, Piura B and Sherbet GV: Metatasis associated mts1 gene expression correlates with increased p53 detection in the B16 murine melanoma cells. DNA Cell Biol *13*: 343-351, 1994.
46 Chen HL, Fernig DG, Rudland PS, Sparks A, Wilkinson MC and Barraclough R: Binding to intracellular targets of the metastasis-inducing protein, S100A (p9Ka). Biochem Biophys Res Commun *286*: 1212-1217, 2001.
47 Cajone F and Sherbet GV: Stathmin is involved in S100A4-mediated regulation of cell cycle progression. Clin Exptl Metastasis *17*: 865-871, 1999.
48 Agoff SN, Hou J, Linzer DIH and Wu B: Regulation of human HSP70 promoter. Science *259*: 84-87, 1993.
49 Tsutsumiishii Y, Todokoro K, Kanaoka F and Tsuchida N: Response of heat shock element within the human HSP70 promoter to mutated p53 genes. Cell Growth Differentiation *6*: 1-8, 1995.
50 He CY, Merrick BA, Patterson RM and Selkirk JK: Altered protein synthesis in p53 null and hemizygotic transgenic mouse embryonic fibroblasts. Appl Theoret Electrophoresis *5*: 15-24, 1995.
51 Sherbet GV and Lakshmi MS: The Genetics of Cancer, Acadmic/Elsevier, London, 1997.
52 Cajone F, Debiasi S, Parker C, Lakshmi MS and Sherbet GV: Metastasis associated mts1 gene is down-regulated by heat shock in variant cell lines of the B16 murine melanoma. Melanoma Res *4*: 143-150, 1994.
53 Sherbet GV, Cajone F, Albertazzi E and Lakshmi MS: Induction of heat shock protein HSP28 and down-regulation of 18A2/mts1 gene are associated with inhibition of proliferation of B16 murine melanoma cell lines. Proc Amer Assoc Cancer Res *37*: 74, 1996.
54 Saito Y, Yamagishi N, Ishihara K and Hatayama T: Identification of alpha-tubulin as an Hsp105 alpha-binding protein by the yeast two-hybrid system. Exp Cell Res *286*: 233-240, 2003.
55 Izawa I, Nishizawa M, Ohtakara K, Ohtsuka K, Inada H and Inagaki M: Identification of Mrj, a DnaJ/Hsp40 family protein, as a keratin 8/18 filament regulatory protein. J Biol Chem *275*: 34521-34527, 2000.
56 Cambiazo V, Gonzalez M, Isamit C and Maccioni RB: The beta-isoform of heat shock protein hsp-90 is structurally related with human microtubule-interacting protein Mip-90. FEBS Lett *457*: 343-347, 1999.
57 Bluhm WF, Martin JL, Mestril R and Dillmann WH: Specific heat shock proteins protect microtubules during simulated ischemia in cardiac myocytes. Am J Physiol Heart Circul Physiol *44*: H2243-H2249, 1998.
58 Gache V, Louwagie M, Garin J, Caudron N, Lafanechere L and Valiron O. Identification of proteins binding the native tubulin dimer. Biochem Biophys Re Commun *327*: 35-42, 2005.
59 Garnier C, Barbier P, Gilli R, Lopez C, Peyrot V and Briand C: Heat-shock protein 90 (hsp90) binds *in vitro* to tubulin dimer and inhibits microtubule formation. Biochem Biophys Res Commun *250*: 414-419, 1998.
60 Fujimoto M, Nagasaka Y, Tanaka T and Nakamura K: Analysis of heat shock-induced monophosphorylation of stathmin in human T lymphoblastic cell line Jurkat by two-dimensional gel electrophoresis. Electrophoresis *19*: 2515-2520, 1998.
61 Beretta L, Duboia MF, Sobel A and Bensaude O: Stathmin is a major substrate for mitogen-activated protein kinase during heat shock and chemical stress. Eur J Biochem *227*: 388-395, 1995.
62 Larsson N, Marklund U, Gradin HM, Brattsand G and Gullberg M: Control of microtubule dynamics by oncoprotein 18. Dissection of the regulatory role of multisite phosphorylation during mitosis. Mol Cell Biol *17*: 5530-5539, 1997.
63 Manceau V, Gavet O, Curmi P and Sobel A: Stathmin interaction with HSC70 family proteins. Electrophoresis *20*: 409-417, 1999.
64 Strey CW, Spellman D, Stieber A, Gonatas JO, Wang XS, Lambris JD and Gonatas NK: Dysregulation of stathmin, a microtubule-destabilizing protein, and up-regulation of Hsp25, Hsp27, and the antioxidant peroxiredoxin 6 in a mouse model of familial amyotrophic lateral sclerosis. Am J Pathol *165*: 1701-1718, 2004.
65 Gomez TM and Letourneau PC: Filopodia initiate choices made by sensory neuron growth cones at laminin/fibronectin borders *in vivo*. J Neurosci *14*: 5959-5972, 1994.
66 Suh LH, Oster SF, Soehrman SS, Grenningloh G and Sretavan DW: L1/laminin modulation of growth cone response to EphB triggers growth pauses and regulates the microtubule destabilizing protein SCG10. J Neurosci *24*: 1976-1986, 2004.

67 Balogh A, Mege RM and Sobel A: Growth and cell density-dependent expression of stathmin in C2 myoblasts in culture. Exp Cell Res *224*: 8-15, 1996.
68 Wittmann T, Bokoch GM and Waterman-Storer CM: Regulation of microtubule destabilizing activity of Op18/ stathmin downstream of Rac1. J Biol Chem *279*: 6196-6203, 2004.
69 Zhou J, Jezova M, Elkahloun AG and Saavedra JM: Angiotensin II AT(1) receptor antagonism downregulates stress-related gene expression in brain microvessels from spontaneously hypertensive and normotensive rats. Ann NY Acad Sci *1018*: 480-486, 2004.
70 Bertrand MJ, Deramaudt M, Remy P and Abraham NG: Upregulation of human heme oxygenase gene expression by Ets-family proteins. J Cell Biochem *72*: 311-321, 1999.
71 Miyashita H, Kanemura M, Yamazaki T, Abe M and Sato Y: Vascular endothelial zinc finger 1 is involved in the regulation of angiogenesis: Possible contribution of stathmin/OP18 as a downstream target gene. Arterioscler Thromb Vasc Biol *24*: 878-884, 2004.
72 Chen G, Wang H, Gharib TG, Huang CC, Thomas DG, Shedden KA, Kuick R, Taylor JMG, Kardia SLR, Misek DE, Giordano TJ, Iannettoni MD, Orringer MB, Hanash SM and Beer DG: Overexpression of oncoprotein 18 correlates with poor differentiation in lung adenocarcinomas. Mol Cell Proteomics *2*: 107-116, 2003.
73 Neben K, Korshunov A, Benner A, Wrobel G, Hahn M, Kokocinski F, Golanov A, Joos S and Lichter P: Microarray-based screening for molecular markers in medulloblastoma revealed STK15 as independent predictor for survival. Cancer Res *64*: 3103-3111, 2004.
74 Balachandran R, Welsh MJ and Day BW: Altered levels and regulation of stathmin in paclitaxel-resistant ovarian cancer cells. Oncogene *22*: 8924-8930, 2003.
75 Alli E, Bash-Babula J, Yang JM and Hait WN: Effect of stathmin on the sensitivity to antimicrotubule drugs in human breast cancer. Cancer Res *62*: 6864-6869, 2002.
76 Nishio K, Nakamura T, Koh Y, Kanzawa F, Tamura T and Saijo N: Oncoprotein 18 overexpression increases the sensitivity to vindesine in the human lung carcinoma cells. Cancer *91*: 1494-1499, 2001.
77 Rosell R, Scagliotti G, Danenberg KD, Lord RVN, Bepler G, Novello S, Cooc J, Crino L, Sanchez JJ, Taron M, Boni C, De Marinis F, Tonato M, Marangolo M, Gozzelino F, Di Costanzo F, Rinaldi M, Salonga D and Stephens C: Transcripts in pretreatment biopsies from a three-arm randomized trial in metastatic non-small-cell lung cancer. Oncogene *22*: 3548-3553, 2003.
78 Mistry SJ, Bank A and Atweh GF: Anti-angiogenesis therapy: targeting vascular endothelial cells by anti-stathmin adenovirus. Blood *102*: 5713, 2003.
79 Iancu C, Mistry SJ, Arkin S and Atweh GF: Taxol and anti-stathmin therapy: a synergistic combination that targets the mitotic spindle. Cancer Res *60*: 3537-3541, 2000.
80 Mistry SJ, Benham CJ and Atweh GF: Development of ribozymes that target stathmin, a major regulator of the mitotic spindle. Antisense Nucleic Acid Drug Dev *11*: 41-49, 2001.

Received May 30, 2005
Accepted June 7, 2005