Published in final edited form as:

J Mol Neurosci. 2008 June ; 35(2): 143–150. doi:10.1007/s12031-008-9044-z.

Effects of Cell Cycle Inhibitors on Tau Phosphorylation in N2aTau3R Cells

Concepcion Conejero-Goldberg,

The Litwin-Zucker Research Center for Study of Alzheimer's Disease, The Feinstein Institute for Medical Research, North Shore University Hospital, 350 Community Drive, Manhasset, NY 11030, USA

Kirk Townsend, and

The Litwin-Zucker Research Center for Study of Alzheimer's Disease, The Feinstein Institute for Medical Research, North Shore University Hospital, 350 Community Drive, Manhasset, NY 11030, USA

Peter Davies

The Litwin-Zucker Research Center for Study of Alzheimer's Disease, The Feinstein Institute for Medical Research, North Shore University Hospital, 350 Community Drive, Manhasset, NY 11030, USA; Department of Pathology, Albert Einstein College of Medicine, Bronx, NY 10461, USA

Concepcion Conejero-Goldberg: cgoldber@nshs.edu

Abstract

Neurofibrillary tangles are one of the pathologic hallmarks of Alzheimer's disease (AD). They are composed of paired helical filaments (PHF) containing hyperphosphorylated forms of tau. Hyperphosphorylation of certain tau sites favors its dissociation from the microtubules (MT), interfering with axonal transport and compromising the function and viability of neurons. Reappearance of cell cycle proteins have been reported in neurons exhibiting tau aggregation, suggesting that an aberrant cell cycle occurs before neurons die. Cell cycle suppression in neurons is crucial to survival, thus prevention of progression through the cell cycle may offer a therapeutic approach. Using a neuroblastoma cell line overexpressing 3-repeat (3R) tau, we investigated the effects of cell cycle inhibitors on tau phosphorylation. G2/M phase inhibitors did not alter phosphorylation of tau at Ser-202 and Ser-396/404 at the lower doses, but did at higher doses. Ser-202 and Ser-396/404 are phosphorylation sites of early and late neurofibrillary tangles, respectively, in AD. Cisplatin, a G1 phase inhibitor, did not phosphorylate tau. Cyclophosphamide and phosphoramidate mustard, DNA cross-linking agents, decreased tau phosphorylation at Ser-396/404 site, but increased phosphorylation at Ser-202. These studies demonstrate that the G2/M blockers have a dose-dependent effect on tau phosphorylation. This seems to be a consequence of both the disruption of MT-organization and MT-dynamics when doses are higher, but only a disruption of MT-dynamics with lower doses. These results are also in agreement with the lack of phosphorylation seen for cisplatin, another inhibitor that produces disruption of the MT-dynamics.

Keywords

Tau; Cell cycle; Alzheimer's disease; Paclitaxel; Vinca alkaloids; Cisplatin; Cyclophosphamide

Introduction

Alzheimer's disease (AD) is the leading cause of dementia worldwide. Over 5 million Americans are afflicted with AD in 2007. Neurofibrillary tangles are one of the pathologic hallmarks of AD. Neurofibrillary tangles are composed of paired helical filaments (PHF) containing hyperphosphorylated forms of the microtubule associated protein, tau. Hyperphosphorylation of certain Ser or Thr phosphorylation tau sites favors its dissociation from the microtubules (MT), leading to destabilization of the neuronal cytoskeleton (Billingsley and Kincaid 1997). Reappearance of cell cycle proteins has been reported in neurons exhibiting tau aggregation, suggesting that an aberrant cell cycle occurs before neurons die (Andorfer et al. 2005). Understanding the mechanisms underlying tau phosphorylation by targeting the cell cycle with compounds that produce cell cycle suppression could provide molecular targets for future therapeutic interventions.

G2/M blockers of the cell cycle such as paclitaxel and Vinca alkaloids (e.g., vinblastine, vincristine) are commonly used as antimitotic drugs (Bhalla 2003; Mollinedo and Gajate 2003; Jordan and Wilson 2004). At lower doses, these drugs have a common mechanism of action; they interfere with polymerization dynamics of the microtubule by hyperstabilization or by destabilization of microtubules and activate the mitotic spindle checkpoint (Blagosklonny and Fojo 1999; Gorbisky 2001; Jordan 2002; Cleveland et al. 2003; Bharadwaj and Yu 2004). The spindle checkpoint causes extended mitotic arrest. During mitosis, the dynamic instability of microtubules has been observed to rise approximately tenfold (Kinoshita et al. 2002). Paclitaxel and the Vinca alkaloids act by suppressing dynamic instability of mitotic spindle microtubules and thus halt cell division at the metaphase/anaphase transition (Jordan 2002). After prolonged periods of mitotic arrest, the cells eventually undergo apoptosis and die either during the mitotic arrest or after cells exit mitosis without normal chromosome segregation (Blagosklonny and Fojo 1999; Weaver and Cleveland 2005).

Cyclophosphamide and phosphoramidate mustard (PM), both DNA cross-linking agents, are capable of inducing apoptosis (Colvin 1999). The main effect of cyclophosphamide is due to its active metabolite PM. This metabolite forms DNA crosslinks between (interstrand) and within (intrastrand) DNA strands at guanine N⁷ positions. Cisplatin is also a DNA cross-linking agent that is most effective in G1 of the cell cycle. It preferentially binds at N⁷ of the purines adenine and guanine, and at N³ of the pyrimidines cytosine and uracil forming DNA crosslinks (90% intrastrand). These DNA cross-linking agents produce alterations of the DNA structure that prevent replication. They also activate cellular repair mechanisms in an attempt to remove by excision the defective section of DNA. Cell cycle checkpoints detect the presence of defective DNA, and trigger a series of responses that lead to apoptotic cell death.

Using a neuroblastoma cell line overexpressing 3-repeat (3R) tau we investigated the effects of cell cycle inhibitor drugs on tau phosphorylation at low and high doses. In these studies, drugs that produce arrest at G2/M phase, like paclitaxel, vincristine (VC), and vinblastine (VBT) increased phosphorylation of tau at Ser-202 and Ser-396/404 at higher doses, but not at lower doses. Cyclophosphamide and PM, both DNA cross-linking agents, decreased tau phosphorylation at Ser-396/404 site, but increased tau phosphorylation at Ser-202. Cisplatin, another DNA cross-linking agent most effective in G1 phase of the cell cycle, did not affect phosphorylation of tau at these sites.

Materials and Methods

Cell Culture

Mouse neuroblastoma N2A cells (provided courtesy of Dr. Philippe Marambaud) were maintained at 37°C and 5% CO₂, in Dulbecco's modified minimal essential medium (DMEM;

Life Technologies, Burlington, ON, Canada) supplemented with 10% (v/v) fetal bovine serum (FBS), 1% (v/v) penicillin and streptomycin (Life Technologies, Burlington, ON). N2A cells were transiently transfected with a 3R human tau construct using GeneCarrier reagent (Epoch Biolabs, TX, USA) and Lipofectamine (Invitrogen, CA, USA) and stable lines were selected with 1 mg/ml medium Geneticin (Invitrogen, CA, USA). These stably transfected N2A cells expressing the 3R human tau were then used in this study and will be referred to as N2aTau3R cells. To differentiate the cells, N2aTau3R cells were treated with retinoic acid (1 μ M) for 24 h in culture medium containing 0.1% FBS. Paclitaxel, vincristine, vinblastine, cisplatin, and cyclophosphamide monohydrate were obtained from Sigma (St. Louis, MO, USA). PM (as the cyclohexylammonium salt) was acquired from the National Cancer Institute.

Protein Isolation and Western Blot Analysis

After incubation with drugs or Dulbecco's modified Eagle medium (DMEM), cells (undifferentiated and differentiated N2a stable-transfected cell cultures) were washed twice with PBS and scraped off the plates in lysis buffer. The lysis buffer composition was: ELB buffer (50 mM HEPES [pH 7.5], 250 mM NaCl, 0.1% Nonidet P-40 [NP-40], 1 mM ethylenediaminetetraacetic acid (EDTA) including protease inhibitors (0.1 mM phenylmethylsulfonyl fluoride [PMSF], 100 μ g of aprotinin, 10 μ g of leupeptin, and 10 μ g of pepstatin per ml, and 1 mM benzamidine). The lysates were centrifuged at 13,500 rpm for 15 min at 4°C, and supernatants were collected. Protein concentration of the cell lysate was determined in each case using the Pierce protein assay kit. Cell lysates were then stored at -20°C. Protein samples (20 μ g protein per lane) were first boiled for 7 min, and then subjected by SDS-PAGE using 8–15% polyacrylamide gels and transferred to nitrocellulose membranes. The nitrocellulose sheets were probed with different antibodies: PHF-1 (Ser-396/404), CP13 (Ser-202) and CP27 (recognizes all human tau isoforms). Polyclonal anti- β -Actin antibody (Santa Cruz Biotechnology, CA, USA) and monoclonal Anti- α -tubulin antibody (Sigma, MO, USA) were used as controls. All nitrocellulose sheets were then labeled with anti-mouse or anti-rabbit antibodies conjugated with peroxidase; visualization was performed using a standard ECL detection procedure.

Results

Induction of Abnormal Phosphorylation of Tau by Paclitaxel at Higher but not at Lower Doses

Paclitaxel, a microtubule-stabilizing agent, is an anti-mitotic drug that induces apoptosis in cancer cells by causing cell cycle arrest at the G2/M transition (Blagosklonny and Fojo 1999; Jordan et al. 1993), activating JNK (Amato et al. 1998; Wang et al. 1998) and inducing phosphorylation of bcl-2 (Blagosklonny et al. 1997; Eisenhauer and Vermorken 1998). The binding of paclitaxel to the β -tubulin subunits in microtubules promotes polymerization of tubulin and disruption of microtubule dynamics, leading to a sustained mitotic arrest and ultimately to apoptotic cell death (Jordan and Wilson 1998).

Paclitaxel interferes with the normal function of microtubule growth by hyperstabilization of their structure. The resulting microtubule/paclitaxel complex does not have the ability to disassemble. This adversely affects cell function because the shortening and lengthening of microtubules (dynamic instability) is necessary for their function as a mechanism to transport other cellular components (e.g., the mitosis where microtubules position the chromosomes during their replication and subsequent separation into the two daughter-cell nuclei). Despite these adverse effects, low doses of paclitaxel may have a therapeutic benefit in human tauopathies by offsetting the loss of normal tau functions that result from its hyperphosphorylation and sequestration into tangles (Zhang et al. 2005).

To further assess the effect of paclitaxel treatment on tau phosphorylation at low and high doses in N2aTau3R cells, we used immunoblot analysis and the CP13, PHF-1 and CP27 tau specific antibodies. CP13 recognizes an epitope phosphorylated at Ser202 and PHF-1 recognizes tau phosphorylated on Ser396/404 (Busciglio et al. 1995). The CP27 antibody recognizes total tau levels independent of phosphorylation state. The N2aTau3R cells under both undifferentiated and differentiated conditions were treated with 10 nM or 1 μ M paclitaxel for 8 h before examining tau phosphorylation.

Phosphorylation of tau protein at both epitopes Ser202 and Ser396/404 was increased at 8 h following incubation with 1 μ M paclitaxel (higher dose). At the lower dose (10 nM), there was no apparent difference in phosphorylation at either Ser202 or Ser396/404 epitope compared to the untreated cells; regardless of the differentiation status of the cells (Fig. 1a,b). In the high-dose (1 μ M) paclitaxel-treated cells, the electrophoretic profile of tau was also modified. This shift in apparent molecular weight associates with hyperphosphorylation of tau. Moreover, the CP27 antibody also recognizes phosphorylated tau species as indicated by the presence of a mobility shift in the tau band from cells treated with paclitaxel. Densitometric quantitation of the CP-13, PHF1, and CP27 immunoreactive bands is shown in (Fig. 1c,d). These results demonstrate that paclitaxel at low dose does not phosphorylate tau, thus opening a window for potential therapeutic treatment at low doses.

Induction of Abnormal Phosphorylation of Tau by Vincristine and Vinblastine at Higher but not at Lower Doses

Vincristine and VBT block the cell cycle in G₂/M phase by depolymerizing microtubules and/or inhibiting their dynamics, eventually leading to cell death (Jordan 2002; Weaver and Cleveland 2005). To assess the effect of VC and VBT treatments on tau phosphorylation in N2aTau3R cells, we used the CP13, PHF-1 and CP27 antibodies. N2aTau3R cells were treated with 1 nM or 1 μ M VC for 24 h for the undifferentiated condition and with 1 nM (data not shown), 1 μ M or 10 μ M VC for the differentiated condition (Fig. 2a,b).

Phosphorylation of tau protein was increased at 24 h after incubation with 1 μ M or 10 μ M VC (higher doses) for both epitopes Ser202 and Ser396/404. At lower dose (1 nM), there was no apparent difference in phosphorylation for either epitope compared to the untreated cells for both conditions, undifferentiated and differentiated. In VC-treated cells with the higher doses (1 μ M or 10 μ M), the electrophoretic profile of tau was modified (Fig. 2a,b). The shift in apparent molecular weight indicates hyperphosphorylation of tau. The CP27 antibody also recognizes phosphorylated tau species as indicated by the presence of a mobility shift in the tau band from cells treated with VC.

Undifferentiated and differentiated N2aTau3R cells were also treated with 1 nM or 1 μ M VBT for 24 h. Phosphorylation of tau protein was clearly increased at 24 h after incubation with 1 μ M VBT for both epitopes Ser202 and Ser396/404 (Fig. 3a,b). However, at lower dose (1 nM) there was no apparent difference in phosphorylation at either epitope when compared to the untreated cells. In the high-dose (1 μ M) VBT-treated cells, the electrophoretic profile of tau was also modified in favor of more tau species (Fig. 3a,b). Densitometric quantitation of the immunoreactive bands for both treatments, VC and VBT, is shown in Figs. 2c,d, and 3c,d, respectively.

Effect of Cisplatin on Tau Phosphorylation

Cisplatin is a DNA cross-linking agent (90% intrastrand crosslinks). Although it is generally known as a phase-nonspecific agent, it may be most effective in G1 of the cell cycle. Cell cycle checkpoints detect the presence of defective DNA, and trigger a series of responses that lead to cellular apoptosis by preventing transcription and upregulation of P53, P21, and Bax.

When either undifferentiated or differentiated N2aTau3R cells were treated with cisplatin (1 nM or 1 μ M) for 24 h, phosphorylation of tau protein at epitopes Ser202 and Ser396/404 was unchanged compared to the untreated cells (Fig. 4a,b). CP27 antibody, which recognizes total tau, showed no differences in the electrophoretic profile between different doses of cisplatin and untreated cells. Densitometry measurements are shown in Fig. 4c and d.

Effect of Cyclophosphamide and Phosphoramidate Mustard on Tau Phosphorylation

Cyclophosphamide and PM are both DNA cross-linking agents that are capable of inducing apoptosis. Oxidative activation of cyclophosphamide by hepatic cytochromes to 4-hydroxycyclophosphamide (4-OH-CPA) leads to spontaneous (non-enzymatic) reactions, ultimately resulting in the production of PM, which is the reactive alkylating agent of therapeutic consequences (Ludeman 1999). The half-life of PM at physiological pH has been reported to be very short and complete loss of free PM should occur within a few hours (Ludeman 1999).

The N2aTau3R cells were treated with 1 mM cyclophosphamide or 1 mM PM for 3 h under undifferentiated and differentiated conditions. For the undifferentiated condition, phosphorylation of tau protein was clearly increased for the Ser202 epitope at 3-h incubation with both cyclophosphamide and PM. For the Ser396/404 epitope, phosphorylation of tau protein was decreased at 3-h incubation with the same treatments. CP27 antibody showed no differences between treated and untreated cells (Fig. 5a). For the differentiated condition, there were no differences between treated and untreated cells in phosphorylation of tau protein as shown by the different antibodies CP13, PHF-1, and CP27 (Fig. 5b). Densitometric quantitation of the CP-13, PHF1, and CP27 immunoreactive bands for these blots is shown in Fig. 5c and d.

Discussion

Tau protein promotes microtubule assembly, but the phosphorylation of certain tau epitopes reduces microtubule binding leading to destabilization of the neuronal cytoskeleton (Billingsley and Kincaid 1997). Reappearance of cell cycle markers have been reported in neurons exhibiting tau aggregation, suggesting that an aberrant cell cycle occurs before neurons die. Prevention of progression through the cell cycle may offer a therapeutic approach if drugs do not induce additional phosphorylation of tau. In the present study, we show that G2/M cell cycle blocker treatments do not induce hyperphosphorylation of tau at lower doses independent of being MT-stabilizing/destabilizing drugs, but induce hyperphosphorylation of tau on Ser 202 and Ser-396/404 consistently at higher doses.

Lower doses of the MT-stabilizing drug paclitaxel could have a therapeutic benefit in human tauopathies by offsetting the loss of normal tau functions that result from its hyperphosphorylation and sequestration into tangles (Zhang et al. 2005). Treatment with paclitaxel restores fast axonal transport in spinal axons, increases axonal MTs, and ameliorates motor impairments in tau transgenic mice that developed filamentous tau inclusions (mostly in the spinal cord) (Ishihara et al. 1999, 2001; Zhang et al. 2005). Paclitaxel also blocks A β -induced phosphorylation of tau by preventing the cleavage of p35 by calpain (Li et al. 2003). Nanomolar concentrations of paclitaxel can protect neurons against various toxic insults and enhance survival by maintaining Ca²⁺ homeostasis (Burke et al. 1994; Furukawa and Mattson 1995; Furukawa et al. 2003; Spone et al. 2003; Michaelis et al. 2005) without evidence of toxicity (Trushina et al. 2003). Our study demonstrates that tau is not phosphorylated by MT-stabilizing/destabilizing drugs at lower doses, but it is phosphorylated at higher doses. Lower doses of these agents do not alter MT organization; meanwhile, higher doses produce disruption of the MT organization (Becvarova et al. 2006). Consequently, MT organization disruption, but not MT-dynamics disruption, is required to promote the hyperphosphorylation of tau.

Adding a layer of complexity, tau phosphorylation may have different effects, depending on site. A recent study analyzed site-specific effects of tau phosphorylation on its microtubule assembly activity and self-aggregation (Liu et al. 2007). Tau phosphorylation at the c-terminal region by glycogen synthase kinase-3 β (GSK-3 β) increased its microtubule assembly activity although promoted its self-aggregation markedly.

Cisplatin, a DNA cross-linking agent, produces alterations of the DNA structure that prevents replication. In addition, it is also able to produce microtubule disassembly by direct tubulin modification (e.g., when platinated, tubulin does not assemble into microtubules, consequently altering MT dynamics) (Boekelheide et al. 1992; Tulub and Stefanov 2001). In our studies, we did not observe tau hyperphosphorylation at the low/high doses used in the study for the treatments with cisplatin. This is also consistent with our results with VC, VBT, and paclitaxel treatments in which tau protein is hyperphosphorylated only when there is alteration of the MT organization but not of the MT-dynamics.

Cyclophosphamide and PM are also DNA cross-linking agents that prevent replication. In our study, both compounds decreased tau phosphorylation at Ser-396/404 when cells were undifferentiated, but increased tau phosphorylation at Ser-202. These contradictory effects may be due to the binding/interfering of the compound with the Ser-396/404 site, so that consequently there is no phosphorylation of tau at that particular site. Another interpretation of these opposing effects on tau phosphorylation between the Ser396/404 and Ser-202 sites could be that cyclophosphamide and its metabolite may have pleiotrophic effects that lead to differentially activation of tau kinases. Clearly, further work is needed to clarify the relationship between DNA cross-linking agents and their effects on microtubule stability/dynamics and how these two phenomena modulate tau phosphorylation. In conclusion, understanding the mechanisms underlying tau phosphorylation by targeting the cell cycle with compounds that produce cell cycle suppression may help to understand why reappearance of cell cycle proteins plays such a critical role in vulnerable neurons. Further studies are needed to provide more answers to the changing of the course of early events of this illness.

Acknowledgments

Phosphoramidate mustard was a gift from the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute.

References

- Amato SF, Swart JM, Berg M, Wanebo HJ, Mehta SR, Chiles TC. Transient stimulation of the c-Jun-NH2-terminal kinase/activator protein 1 pathway and inhibition of extracellular signal-regulated kinase are early effects in paclitaxel-mediated apoptosis in human B lymphoblasts. *Cancer Research* 1998;58(2):241–247. [PubMed: 9443400]
- Andorfer C, Acker CM, Kress Y, Hof PR, Duff K, Davies P. Cell-cycle reentry and cell death in transgenic mice expressing nonmutant human tau isoforms. *Journal of Neuroscience* 2005;25(22):5446–5454. [PubMed: 15930395]
- Becvarova P, Skorpikova J, Janisch R, Novy J. A Vinca alkaloid effect on microtubules of HeLa cells. *Scripta Medica (BRNO)* 2006;79(1):19–34.
- Bhalla KN. Microtubule-targeted anticancer agents and apoptosis. *Oncogene* 2003;22(56):9075–9086. [PubMed: 14663486]
- Bharadwaj R, Yu H. The spindle checkpoint, aneuploidy, and cancer. *Oncogene* 2004;23(11):2016–2027. [PubMed: 15021889]
- Billingsley ML, Kincaid RL. Regulated phosphorylation and dephosphorylation of tau protein: effects on microtubule interaction, intracellular trafficking and neurodegeneration. *Biochemical Journal* 1997;323(Pt 3):577–591. [PubMed: 9169588]

- Blagosklonny MV, Fojo T. Molecular effects of paclitaxel: myths and reality (a critical review). *International Journal of Cancer* 1999;83(2):151–156.
- Blagosklonny MV, Giannakakou P, el-Deiry WS, et al. Raf-1/bcl-2 phosphorylation: a step from microtubule damage to cell death. *Cancer Research* 1997;57(1):130–135. [PubMed: 8988053]
- Boekelheide K, Arcila ME, Eveleth J. Cis-diamminedi-chloroplatinum (II) (cisplatin) alters microtubule assembly dynamics. *Toxicology & Applied Pharmacology* 1992;116(1):146–151. [PubMed: 1529448]
- Burke WJ, Raghu G, Strong R. Taxol protects against calcium-mediated death of differentiated rat pheochromocytoma cells. *Life Sciences* 1994;55(16):313–319. [PubMed: 7934633]
- Busciglio J, Lorenzo A, Yeh J, Yankner BA. Beta-amyloid fibrils induce tau phosphorylation and loss of microtubule binding. *Neuron* 1995;14(4):879–888. [PubMed: 7718249]
- Cleveland DW, Mao Y, Sullivan KF. Centromeres and kinetochores: from epigenetics to mitotic checkpoint signaling. *Cell* 2003;112(4):407–421. [PubMed: 12600307]
- Colvin OM. An overview of cyclophosphamide development and clinical applications. *Current Pharmaceutical Design* 1999;5(8):555–560. [PubMed: 10469891]
- Eisenhauer EA, Vermorken JB. The taxoids. *Comparative clinical pharmacology and therapeutic potential*. *Drugs* 1998;55(1):5–30. [PubMed: 9463787]
- Furukawa K, Mattson MP. Taxol stabilizes $[Ca^{2+}]_i$ and protects hippocampal neurons against excitotoxicity. *Brain Research* 1995;689(1):141–146. [PubMed: 8528698]
- Furukawa K, Wang Y, Yao PJ, et al. Alteration in calcium channel properties is responsible for the neurotoxic action of a familial frontotemporal dementia tau mutation. *Journal of Neurochemistry* 2003;87(2):427–436. [PubMed: 14511120]
- Gorbsky GJ. The mitotic spindle checkpoint. *Current Biology* 2001;11(24):R1001–R1004. [PubMed: 11747833]
- Ishihara T, Hong M, Zhang B, et al. Age-dependent emergence and progression of a tauopathy in transgenic mice overexpressing the shortest human tau isoform. *Neuron* 1999;24(3):751–762. [PubMed: 10595524]
- Ishihara T, Zhang B, Higuchi M, Yoshiyama Y, Trojanowski JQ, Lee VM. Age-dependent induction of congophilic neurofibrillary tau inclusions in tau transgenic mice. *American Journal of Pathology* 2001;158(2):555–562. [PubMed: 11159192]
- Jordan MA. Mechanism of action of antitumor drugs that interact with microtubules and tubulin. *Current Medicinal Chemistry Anti-Cancer Agents* 2002;2(1):1–17. [PubMed: 12678749]
- Jordan MA, Toso RJ, Thrower D, Wilson L. Mechanism of mitotic block and inhibition of cell proliferation by taxol at low concentrations. *Proceedings of the National Academy of Sciences of the United States of America* 1993;90(20):9552–9556. [PubMed: 8105478]
- Jordan MA, Wilson L. Microtubules and actin filaments: dynamic targets for cancer chemotherapy. *Current Opinion in Cell Biology* 1998;10(1):123–130. [PubMed: 9484604]
- Jordan MA, Wilson L. Microtubules as a target for anticancer drugs. *Nature Reviews Cancer* 2004;4(4):253–265.
- Kinoshita K, Habermann B, Hyman AA. XMAP215: a key component of the dynamic microtubule cytoskeleton. *Trends in Cell Biology* 2002;12(6):267–273. [PubMed: 12074886]
- Li G, Faibushevich A, Turunen BJ, et al. Stabilization of the cyclin-dependent kinase 5 activator, p35, by paclitaxel decreases beta-amyloid toxicity in cortical neurons. *Journal of Neurochemistry* 2003;84(2):347–362. [PubMed: 12558997]
- Liu F, Li B, Tung EJ, Grundke-Iqbal I, Iqbal K, Gong CX. Site-specific effects of tau phosphorylation on its microtubule assembly activity and self-aggregation. *European Journal of Neuroscience* 2007;26(12):3429–3436. [PubMed: 18052981]
- Ludeman SM. The chemistry of the metabolites of cyclophosphamide. *Current Pharmaceutical Design* 1999;5(8):627–643. [PubMed: 10469895]
- Michaelis ML, Ansar S, Chen Y, et al. {beta}-Amyloid-induced neurodegeneration and protection by structurally diverse microtubule-stabilizing agents. *Journal of Pharmacology and Experimental Therapeutics* 2005;312(2):659–668. [PubMed: 15375176]
- Mollinedo F, Gajate C. Microtubules, microtubule-interfering agents and apoptosis. *Apoptosis* 2003;8(5):413–450. [PubMed: 12975575]

- Sponne I, Fifre A, Drouet B, et al. Apoptotic neuronal cell death induced by the non-fibrillar amyloid-beta peptide proceeds through an early reactive oxygen species-dependent cytoskeleton perturbation. *Journal of Biological Chemistry* 2003;278(5):3437–3445. [PubMed: 12435748]
- Trushina E, Heldebrant MP, Perez-Terzic CM, et al. Microtubule destabilization and nuclear entry are sequential steps leading to toxicity in Huntington's disease. *Proceedings of the National Academy of Sciences of the United States of America* 2003;100(21):12171–12176. [PubMed: 14527999]
- Tulub AA, Stefanov VE. Cisplatin stops tubulin assembly into microtubules. A new insight into the mechanism of antitumor activity of platinum complexes. *International Journal of Biological Macromolecules* 2001;28(3):191–198. [PubMed: 11251225]
- Wang TH, Wang HS, Ichijo H, et al. Microtubule-interfering agents activate c-Jun N-terminal kinase/stress-activated protein kinase through both Ras and apoptosis signal-regulating kinase pathways. *Journal of Biological Chemistry* 1998;273(9):4928–4936. [PubMed: 9478937]
- Weaver BA, Cleveland DW. Decoding the links between mitosis, cancer, and chemotherapy: the mitotic checkpoint, adaptation, and cell death. *Cancer Cell* 2005;8(1):7–12. [PubMed: 16023594]
- Zhang B, Maiti A, Shively S, et al. Microtubule-binding drugs offset tau sequestration by stabilizing microtubules and reversing fast axonal transport deficits in a tauopathy model. *Proceedings of the National Academy of Sciences of the United States of America* 2005;102(1):227–231. [PubMed: 15615853]

Figure 1.

Paclitaxel (Px) phosphorylates tau at Ser-202 and Ser-396/404 in N2a cells overexpressing 3R tau at the higher doses but not at lower doses. **(a)** Undifferentiated mouse neuroblastoma cells overexpressing 3R tau protein (N2aTau3R cells) and treated for 8 h with 0.01 or 1 μM concentrations of Px. **(b)** N2aTau3R cells are differentiated with retinoid acid (RA) for 24 h and treated as described in A. Blots are performed using anti-Ser 202 antibody (CP13), anti-Ser396/404 antibody (PHF1), and the CP27 antibody, which recognizes total tau levels. **(c)** Densitometry values for the undifferentiated cells Px-treated, and **(d)** densitometry for the differentiated cells. Western blots are representative of two independent experiments

Figure 2.

Vincristine (VC) phosphorylates tau at Ser-202 and Ser-396/404 in N2a cells overexpressing 3R tau at higher doses but not at lower doses. **(a)** Undifferentiated N2aTau3R cells are treated for 24 h with 0.001 or 1 μ M concentrations of VC. **(b)** N2aTau3R cells are differentiated with RA for 24 h and treated for 24 h with 1.0 or 10 μ M concentrations of VC. Blots are performed using anti-Ser 202 antibody (CP13), anti-Ser396/404 antibody (PHF1), and the CP27 antibody, which recognizes total tau levels. **(c)** Densitometry values for the undifferentiated cells treated with VC, and **(d)** densitometry for the differentiated cells. Western blots are representative of two independent experiments

Figure 3.

Vinblastine (VBT) phosphorylates tau at Ser-202 and Ser-396/404 in N2a cells overexpressing 3R tau at higher doses but not at lower doses. **(a)** Undifferentiated N2aTau3R cells are treated for 24 h with 0.001 or 1 μ M concentrations of VBT. **(b)** N2aTau3R cells are differentiated with RA for 24 h and treated as described in A. Blots are performed using anti-Ser 202 antibody (CP13), anti-Ser396/404 antibody (PHF1), and the CP27 antibody, which recognizes total tau levels. **(c)** Densitometry values for the undifferentiated cells treated with VBT, and **(d)** densitometry for the differentiated cells. Western blots are representative of two independent experiments

Figure 4.

Cisplatin (CP) does not phosphorylate tau at Ser-202 and Ser-396/404 in N2a cells overexpressing 3R tau. **(a)** Undifferentiated N2aTau3R cells are treated for 24 h with 0.001 or 1 μ M concentrations of cisplatin. **(b)** N2aTau 3R cells are differentiated with RA for 24 h and treated as described in A. Blots are performed using anti-Ser 202 antibody (PC13), anti-Ser396/404 antibody (PHF1), and the CP27 antibody, which recognizes total tau levels. **(c)** Densitometry values for the undifferentiated cells treated with CP, and **(d)** densitometry for the differentiated cells. Western blots are representative of two independent experiments

Figure 5. Effect of cyclophosphamide (Cyclo) and phosphoramidate mustard (PM) on tau phosphorylation at Ser-202 and Ser-396/404 in N2a cells overexpressing 3R tau. **(a)** Undifferentiated N2aTau3R cells are treated for 3 h with 1-mM concentrations of Cyclo and PM. Both compounds increase tau phosphorylation at Ser 202 site, but decrease tau phosphorylation at Ser396/404 site. **(b)** N2aTau3R cells are differentiated with RA for 24 h and treated as described in **A**. Cyclo and PM do not produce tau phosphorylation at the Ser202 or Ser396/404 site. Blots are performed using anti-Ser 202 antibody (PC13), anti-Ser396/404 antibody (PHF1), and the CP27 antibody, which recognizes total tau levels. **(c)** Densitometry values for the undifferentiated cells treated with Cyclo-PM and **D**, densitometry for the differentiated cells. Western blots are representative of two independent experiments