Nordihydroguaiaretic Acid Affects Multiple Dynein-Dynactin Functions in Interphase and Mitotic Cells

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ABSTRACT

Nordihydroguaiaretic acid (NDGA), a well known lipoxygenase inhibitor, actually has pleiotropic effects on cells, which include cell proliferation, apoptosis, differentiation, and chemotaxis. We and others have shown previously that this compound causes Golgi disassembly by an unknown mechanism. In this study, we show that, in parallel with Golgi disassembly, NDGA induces the accumulation of the microtubule minus-end-directed motor dynein-dynactin complex at the centrosome, where microtubules minus-ends lie. Concomitant with this accumulation, dynein-dynactin–interacting proteins, such as ZW10 and EB1, were also redistributed to the centrosomal region. In cells where microtubules were depolymerized by nocodazole, NDGA promoted the formation of filaments consisting of dynein-dynactin and its interacting proteins, suggesting that it stimulates the association of these proteins in an ordered, not random, manner. Loss of dynactin function abolished not only NDGA-induced redistribution in intact cells but also filament formation in nocodazole-treated cells. The latter finding implies that dynactin is a key molecule for the association between dynein-dynactin and its interacting proteins. In mitotic cells, NDGA induced robust accumulation of dynein-dynactin and its interacting proteins at the spindle poles. These results taken together suggest that NDGA perturbs membrane traffic by affecting the function of the microtubule motor dynein-dynactin complex and its auxiliary proteins. To our knowledge, NDGA is the first case of a reagent that can modulate dynein-dynactin–related processes.

Nordihydroguaiaretic acid (NDGA) is a drug that affects a wide variety of cellular processes, including growth factor- and tumor necrosis factor-induced signal transduction (Domin et al., 1994; Lee et al., 2003; West et al., 2004), leukocyte chemotaxis (Goetzl, 1980), myoblast cell differentiation (Ito et al., 2005), cancer cell proliferation (Avis et al., 1996; McDonald et al., 2001; Seufferlein et al., 2002; Youngren et al., 2005), and viral proliferation in infected cells (Gnabre et al., 1995). It can also induce nitric-oxide synthase expression (Ramasamy et al., 1999), regulate calcium channel activity (Korn and Horn, 1990; Huang et al., 2004), and inhibit growth of β-amyloid (1–40) protofibril (Moss et al., 2004). Although many of the effects of NDGA on cellular events seem to be ascribable to its action as a lipoxygenase inhibitor or an antioxidant, some are obviously peculiar to the action of NDGA (Korn and Horn, 1990; Lee et al., 2003; Huang et al., 2004; Ito et al., 2005). The anticancer activity of NDGA may be due in part to its inhibition of protein kinase C and receptor tyrosine kinases (Domin et al., 1994; Youngren et al., 2005).

We have shown for the first time that NDGA perturbs intracellular membrane traffic. NDGA inhibits the vesicle-mediated transport of vesicular stomatitis virus-encoded glycoprotein both within the Golgi apparatus in vitro (Tagaya et al., 1993) and from the endoplasmic reticulum (ER) to the Golgi in vivo (Tagaya et al., 1996). Later studies demonstrated that NDGA induces Golgi disassembly (Yamaguchi et al., 1999), which leads Golgi components to be redistributed to the ER (Drecketrah et al., 1998; Fujiwara et al., 1998a). In addition to the secretory pathway, this compound blocks the endocytic pathway in human dendritic cells in a manner independent of inhibition of lipoxygenases and prevention of reactive oxygen species formation (Ramoner et al., 1998), raising the possibility that NDGA affects the machinery generally required for vesicular transport but not that for specific transport processes. In this context, the finding of Na-
kamura et al. (2003) that showed that NDGA is capable of stabilizing microtubules (MTs) is worth noting, because they generally participate in membrane transport along the secretory and endocytic pathways. However, the relationship between the two NDGA-induced effects, MT stabilization and transport defect, is totally unknown.

We have recently demonstrated that, in interphase cells, ZW10 is present in the ER membrane, as well as in the cytosol, and plays a role in membrane traffic between the ER and Golgi (Hirose et al., 2004). ZW10 was originally characterized as a kinetochore-associated component that interacts with dynamitin (Starr et al., 1998), a subunit of dynactin that provides a link between the MT minus-end–directed motor dynein and cargo molecules. In the course of our study on ZW10, we found that NDGA induces the accumulation of ZW10 at the centrosome, where MT minus-ends lie. To better understand the mechanism for NDGA-induced Golgi disassembly, we examined in detail how NDGA induces ZW10 redistribution. We found that NDGA affects dynein-dynactin such that this motor associates more tightly with ZW10. The enhanced association may allow a long-range movement of dynein-dynactin and its interacting proteins toward the centrosome, which leads to imbalance in membrane traffic, thereby causing Golgi disassembly.

Materials and Methods

Antibodies. Monoclonal antibodies (Abs) against dynamitin, EB1 and p150\(^{Gluad}\) were obtained from Transduction Laboratory. Monoclonal Abs against dynactin intermediate chain (IC) and \(\gamma\)-tubulin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA) and Accurate Chemical & Scientific (Westbury, NY), respectively. The preparation and sources of other Abs were described previously (Yoshimori et al., 1988; Hirose et al., 2004).

Chemicals. Ascorbic acid, \(N\)-acetyl cysteine, \(\alpha\)-tocopherol, propidium iodide, and paclitaxel (Taxol) were obtained from Wako Pure Chemicals (Osaka, Japan). Nocodazole (Noc) and 5,8,11,14-eicosatetraenoic acid were obtained from Sigma (St. Louis, MO). NDGA was purchased from BIOMOL Research Laboratories (Plymouth Meeting, PA) and freshly dissolved in dimethyl sulfoxide (DMSO) before use. When cells were incubated with these reagents, fetal calf serum was omitted from culture medium. Transferrin (TF)-fluorescein isothiocyanate (FITC) was purchased from Invitrogen (Carlsbad, CA).

Plasmid, Cell Culture, and Transfection. The full-length cDNA of dynamitin was inserted into pFLAG-CMV2. HeLa cells were cultured in Eagle's minimum essential medium supplemented with 50 IU/ml penicillin, 50 \(\mu\)g/ml streptomycin, and 10% fetal calf serum. Transfection of cells with plasmids was performed according to the manufacturer's protocol using Lipofectamine PLUS reagent (Invitrogen). Double thymidine block was performed as described by Chan et al. (2000).

Immunoprecipitation and Immunofluorescence. Preparations of cell lysates and immunoprecipitation were performed as described previously (Hirose et al., 2004). Immunofluorescence microscopy was performed as described previously (Hirose et al., 2004). Unless otherwise stated, cells were fixed with methanol at \(-20^\circ\)C for 5 min.

Semi-Intact Cells. To express ZW10-GFP, Tet-on inducible ZW10-GFP expression cells (Hirose et al., 2004) were incubated with 1.0 \(\mu\)g/ml doxycycline for 48 h. The cells were washed twice with permeabilization buffer (25 mM HEPES-KOH, pH 7.4, 115 mM potassium acetate, 2.5 mM MgCl\(_2\), 2 mM EGTA, and 1 mg/ml glucose), and then permeabilized with 40 \(\mu\)g/ml digitonin in the same buffer at 0\(^\circ\)C for 5 min. After washing with permeabilization buffer twice, the cells were incubated in 1 ml of the reaction mixture at 32\(^\circ\)C for 1 h. The reaction mixture contained permeabilization buffer plus an ATP-regenerating system (8 mM creatine phosphate, 1 mM ATP, and 50 \(\mu\)g/ml creatine kinase) and 2.5 mg/ml rat liver cytosol.

RNA Interference. Duplex RNAs for targeting were ZW10 (102) (5'-AAGGGTGAGGGTGGCAATGTG-3') and p150\(^{Gluad}\) (207) (5'-TATGGAATCTTCAAGGC-3'). They were purchased from Japan Bioservice (Asaka, Japan). RNA interference experiments were conducted as described previously (Hirose et al., 2004).

Uptake and Recycling of Tf. Uptake and recycling experiments were conducted as described previously (Hirose et al., 2004) with a slight modification.

Results

NDGA Caused Redistribution of ZW10 to the Centrosomal Region. Upon incubation of HeLa cells with 30 \(\mu\)M NDGA for 1 h, ZW10 was redistributed from the ER to the perinuclear area in parallel with dispersion of a Golgi marker protein, GM130 (Fig. 1A, +NDGA, top and middle rows). The perinuclear region where ZW10 was accumulated was marked by \(\gamma\)-tubulin, a centrosome marker (bottom row).

To gain insight into the mechanism for NDGA-induced ZW10 redistribution, we first examined whether this redistribution is caused by scavenging of reactive oxygen species or inhibiting lipoxygenases. Cells were incubated with an antioxidant (\(\alpha\)-tocopherol, ascorbic acid, or \(N\)-acetyl cysteine) or a lipoxygenase inhibitor (5,8,11,14-eicosatetraynoic acid) for a prolonged time, and then the distribution of ZW10 was analyzed. As shown in Fig. 1B, none of the reagents induced the redistribution of ZW10 to the centrosomal region. In addition, paclitaxel, a well known MT-stabilizing reagent, did not cause ZW10 redistribution. These results indicate that ZW10 redistribution to the centrosomal region was not due to the prevention of reactive oxygen species formation, inhibition of lipoxygenases, or stabilization of MTs.

To determine whether energy and/or cytosolic factors are necessary for NDGA-induced ZW10 redistribution, we used cells expressing ZW10-GFP. ZW10-GFP–expressing cells were permeabilized to remove the cytosol (including cytosolic ZW10-GFP), and then NDGA, together with ATP and rat liver cytosol, was added. As shown in Fig. 1C, ZW10 was translocated to the centrosomal region only when all components were present, implying that ATP and cytosolic component(s) are required for ZW10 redistribution. In addition, this result confirmed that the ER-associated form of ZW10 is translocated.

ZW10 Redistribution Was Driven by Dynein-Dynactin. Given that ZW10 interacts with dynamitin (Starr et al., 1998), a subunit of dynactin, the most straightforward interpretation of the results described above is that NDGA induces ZW10 redistribution to the centrosome by facilitating its minus-end–directed movement driven by the MT motor dynein-dynactin complex. This idea was supported by the observation that a subunit of the dynactin, p150\(^{Gluad}\), and dynein IC also accumulated at the centrosomal region upon NDGA treatment (Fig. 2A, +NDGA).

To verify that dynein-dynactin mediates the NDGA-induced movement of ZW10 to the centrosomal region, we first examined the effect of overexpression of dynamitin. Overexpression of dynamitin is known to block dynein-dynactin–mediated processes by disassembling the dynactin complex (Burkhardt et al., 1997). The plasmid for FLAG-dynamitin or, as a control, DsRed, was transfected into HeLa cells; 24 h
after transfection, the cells were treated with NDGA. As shown in Fig. 2B, NDGA-induced ZW10 redistribution was almost completely inhibited by dynamitin overexpression but not by DsRed overexpression.

Next, we examined the effect of knockdown of p150Glued on NDGA-induced ZW10 redistribution. HeLa cells were transfected with a short interfering RNA named p150Glued (207) or ZW10 (102) and incubated for 72 h. Immunoblotting revealed that the expression levels of p150Glued and ZW10 were markedly reduced (Fig. 2C). In p150Glued-depleted cells, ZW10 did not accumulate at the centrosomal region upon NDGA treatment [Fig. 2C, p150Glued (207)], suggesting that dynactin function might be required for NDGA-induced ZW10 redistribution. It should be noted that depletion of ZW10 did not affect the accumulation of p150Glued at the centrosome (Fig. 2C, ZW10 (102)). This implies that dynactin is required for the redistribution of ZW10 but not vice versa.

NDGA Seemed to Stimulate the Association of Dynactin with ZW10. Consistent with the idea that NDGA-induced redistribution of ZW10 is mediated by dynein-dynactin, ZW10 was not accumulated at the centrosomal region

Fig. 1. Characterization of NDGA-induced ZW10 redistribution. A, HeLa cells were treated for 1 h with 0.1% DMSO (Vehicle) or 30 μM NDGA (+NDGA) and then double stained with Abs against ZW10 and GM130 (top and middle rows) or γ-tubulin (bottom row). N denotes the position of the nucleus. Scale bar, 5 μm. B, HeLa cells were treated with 0.1% DMSO (Vehicle) for 1 h, 20 μM α-tocopherol for 5 h, 1 mM ascorbic acid for 5 h, 1 mM N-acetyl cysteine (NAC) for 5 h, 20 μM 5,8,11,14-eicosatetraynoic acid (ETYA) for 3 h, or 10 μg/ml paclitaxel for 2 h. Fetal calf serum was omitted during incubation with these reagents. The cells were stained with an Ab against ZW10. Scale bar, 5 μm. C, requirement of energy and cytosolic factor(s) for ZW10 redistribution. Digitonin-permeabilized HeLa cells expressing ZW10-GFP were incubated at 32°C for 60 min without or with 30 μM NDGA in the presence of cytosol (Cy) and/or an ATP-regenerating system (ATP). Arrows indicate the position of centrosomal ZW10-GFP. Scale bar, 5 μm. The quantitative results are shown on the right. Error bars represent the S.E.M. for three experiments.

Fig. 2. Dynactin function is required for NDGA-induced ZW10 redistribution. A, HeLa cells were treated with 0.1% DMSO (Vehicle) or 30 μM NDGA (+NDGA) for 1 h and then double-stained with Abs against ZW10 and p150Glued or dynein IC. Scale bar, 5 μm. B, HeLa cells were transfected with the plasmid for FLAG-dynamitin or DsRed. After 24 h, the cells were treated with 30 μM NDGA for 1 h and then double stained with Abs against ZW10 and FLAG (upper row) or only stained for ZW10 (lower row). Although methanol treatment failed to fix most of the expressed DsRed, DsRed-expressing cells were recognizable because of the presence of DsRed remnants. Scale bar, 5 μm. The quantitative results are shown on the right. Error bars represent the S.E.M. for three experiments. C, HeLa cells were transfected without (Mock) or with p150Glued (207) or ZW10 (102). At 72 h after transfection, the cells were solubilized in phosphate-buffered saline with 0.5% SDS and analyzed by immunoblotting. Alternatively, the transfected cells were incubated for 1 h with 0.1% DMSO (Vehicle) or 30 μM NDGA (+NDGA) and then double-stained with Abs against p150Glued and ZW10. Scale bar, 5 μm.
when MTs were depolymerized by Noc before NDGA treatment. It is noteworthy that, in Noc- and NDGA-treated cells, ZW10 exhibited filamentous structures at the cell periphery. These filaments were positive for the dynactin subunit p150Glued (Fig. 3A) but negative for MTs (data not shown). The formation of these filaments could imply that NDGA stimulates the association between ZW10 and dynactin in a manner that forms an ordered structure. To test whether NDGA influences the association between these proteins, we performed immunoprecipitation using an anti-ZW10 Ab. As shown in Fig. 3A, the amounts of p150Glued and dynein IC coprecipitated with ZW10 were increased in the presence of NDGA, whereas γ-tubulin, which was used as a negative control, was not coprecipitated with ZW10 regardless of whether NDGA was present or not.

To obtain evidence that dynactin is required for the formation of ZW10-positive filaments, the expression of p150Glued was suppressed by RNA interference, and then NDGA was added to cells with depolymerized MTs. As shown in Fig. 3B, no obvious ZW10 filaments were observed in p150Glued-depleted cells (middle row), whereas p150Glued-positive filamentous structures were detected in ZW10-depleted cells (bottom row).

**NDGA Induced Centrosomal Accumulation of EB1.** Is the action of NDGA specific for ZW10? Alternatively, do other dynactin-interacting proteins also undergo redistribution in the presence of NDGA? To address this question, we investigated the effect of NDGA on the localization of EB1, a MT plus-end tracking protein that is known to interact directly with p150Glued (Berrueta et al., 1999). Without NDGA treatment, EB1 displayed a “comet tail” pattern, representing its predominant association with the growing ends of MTs (Fig. 4, Vehicle). Upon incubation of cells with NDGA, EB1 as well as ZW10 accumulated at the centrosomal region (Fig. 4, +NDGA). As observed for ZW10, NDGA-induced EB1 redistribution was prevented by dynamitin overexpression or depletion of p150Glued and the formation of EB1 filaments observed in Noc-treated cells was dependent on the presence of p150Glued (data not shown).

**NDGA Induced Robust Accumulation of Dynein-Dynactin and Its Interacting Proteins at the Spindle Poles.** During mitosis, dynein-dynactin and its interacting proteins, including ZW10 and EB1, participate in the organization of spindles, spindle checkpoint, and segregation of chromosomes (Chan et al., 2000; Green et al., 2005). We investigated whether the distributions of dynein-dynactin and its interacting proteins in mitotic cells are affected by NDGA, as observed in interphase cells. Incubation of mitotic cells with NDGA resulted in robust accumulation of these proteins at the spindle poles (Fig. 5A, +NDGA). It was remarkable that almost all cells treated with NDGA displayed a metaphase-like pattern. Moreover, a significant fraction of NDGA-treated cells showed aberrant chromosome distribution. In addition to being localized at the metaphase plate, chromosomes were localized at or near spindle poles (Fig. 5B, +NDGA) or outside of the spindles (data not shown).

**Effect of NDGA on the Distribution of Tf Receptor.** Dynein-dynactin is present on endosomes (Habermann et al., 2000) and endocytosis of Tf is blocked by disruption of dynein-dynactin function (Burkhardt et al., 1997). Because NDGA inhibits the endocytic pathway (Ramoner et al., 1998), we examined whether the localization of TfR is affected by NDGA. In control cells, TfR was distributed throughout the cytoplasm with some concentration in the perinuclear region, which may represent recycling endosomes (Fig. 6A, Vehicle). Upon incubation with NDGA, TfR accumulated in the perinuclear region with a marked loss of peripheral localization (Fig. 6A, +NDGA). Consistent with a previous study (Ramoner et al., 1998), uptake of Tf-FITC was blocked in the
presence of NDGA (Fig. 6B, +NDGA). Furthermore, recycling of Tf-FITC (i.e., release of incorporated Tf-FITC into the medium) was also blocked by NDGA, and the Tf-FITC remained colocalized with TfR in the perinuclear region (Fig. 6C, +NDGA). This phenotype is in marked contrast to that in cells overexpressing dynamin. In dynamin-overexpressing cells, uptake of Tf occurs but its movement to the cell center is blocked (Burkhardt et al., 1997).

Uptake and recycling of Tf are arrested during mitosis (Sager et al., 1984). We were interested in whether TR distribution in mitotic cells was affected by NDGA. In mitotic cells, TfR was found to be distributed diffusely throughout the cell with some concentration at the spindle poles (Fig. 6D, Vehicle). In marked contrast to the case of interphase cells, addition of NDGA did not significantly affect the localization of TfR (Fig. 6D, +NDGA). This may imply that the connection between TfR-containing endosomes and dynein-dynactin is regulated in a cell cycle-dependent manner. Indeed, previous work demonstrated that dynein-dynactin detaches from membranes in metaphase Xenopus laevis egg extracts (Niclas et al., 1996).

**Discussion**

In the present study, we demonstrated that NDGA induces the accumulation of dynein-dynactin at the centrosome in interphase cells and at the spindle poles in mitotic cells. Concomitant with the movement of dynein-dynactin, dynactin-interacting proteins, such as ZW10 and EB1, were also transported, depending on cell cycle, to the centrosomal region or the spindle poles. Upon NDGA treatment, TfR was also redistributed to the centrosomal region in interphase cells. Loss of dynactin function caused by disassembly of dynactin by dynamin overexpression or depletion of the dynactin subunit p150^Glued^ abrogated the NDGA-induced movement of dynactin-interacting components to the centrosomal region, whereas depletion of ZW10 had no effect on NDGA-induced redistribution of dynein-dynactin. These results suggest that NDGA can affect dynein-dynactin function and facilitate processes mediated by this motor.

**The Mechanism for NDGA Action on Dynein-dynactin and Its Interacting Proteins.** How can NDGA induce the movement of dynein-dynactin and its interacting proteins to the centrosome? The phenotype of cells with depolymerized MTs provides a clue to understanding the mechanism for this NDGA action. When MTs were depolymerized, NDGA promoted the formation of filaments comprising dynein-dynactin, ZW10, and EB1. The filament formation in the absence of intact MTs most likely reflects the enhanced association between dynein-dynactin and its interacting proteins. Perhaps the NDGA-induced association of these proteins is highly ordered because NDGA treatment of Noc-treated cells did not induce aggregation, which is a hallmark of nonspecific protein-protein interactions. The result of immunoprecipitation analysis supported the view that NDGA strengthens the association between dynein-dynactin and ZW10. Given that dynactin functions not only as a cargo adaptor but also as a factor conferring dynein processivity (Culver-Hanlon et al., 2006), it is tempting to speculate that the enhanced association between dynein-dynactin and its interacting proteins allows a long-range movement of these proteins toward the minus-end of MTs, leading to their accumulation at the centrosomal region.

**Mechanism of Golgi Disassembly and Endocytosis Inhibition by NDGA.** Our results suggest that the Golgi disassembly and blockage of endocytosis induced by NDGA are due to the excessive stimulation of dynein-dynactin-mediated processes. This view can explain the finding by Fujiwara et al. (1998b) that, upon NDGA treatment, a marker for the ER-Golgi intermediate compartment, ERGIC-53, rapidly moves to the perinuclear, centrosomal region before Golgi enzymes to be redistributed to the ER. This movement of ERGIC-53 is probably coupled to the movement of dynein-dynactin to the centrosomal region. The ER-Golgi intermediate compartment coalescences with the Golgi to form aggregated membrane structures, which may fuse directly with proximal ER membranes (Fujiwara et al., 2003). In the case of endocytosis, TFR-containing endosomes, in association with dynein-dynactin, accumulate at the centrosomal region, leading to a deficiency of TfR at the plasma membrane.

Based on the observation that activation of trimeric GTP-binding proteins prevents NDGA-induced Golgi disassembly, we previously suggested that this reagent affects the function of trimeric GTP-binding proteins (Yamaguchi et al., 1997). However, as dynein-dynactin and its interacting proteins accumulated at the centrosomal region upon NDGA treatment irrespective of the presence or absence of aluminum fluoride (data not shown), the previous interpretation should be modified. Perhaps, activation of GTP-binding proteins stabilizes Golgi membranes by extensively recruiting peripheral protein complexes to the membranes so that lateral movement of Golgi membrane proteins is constrained (Cole et al., 1996). The stabilized Golgi apparatus can maintain its structure even when large amounts of dynein-dynactin and its interacting proteins accumulate at the centrosomal region.

**Fig. 4. Distribution of EB1 in NDGA-treated cells.** HeLa cells were treated for 1 h with 0.1% DMSO (Vehicle) or 30 μM NDGA (+NDGA) and then double-stained with Abs against EB1 and ZW10. Scale bar, 5 μm.
NDGA as a Drug to Regulate MT Stability and MT-related Processes. NDGA, unlike paclitaxel, does not promote MT polymerization, although it stabilizes MTs (Nakamura et al., 2003). Based on the result of an indirect measurement, Nakamura et al. (2003) suggested that NDGA prevents MT depolymerization by directly binding to tubulin. However, no tubulin was found in the filaments formed upon NDGA treatment of Noc-treated cells, suggesting that the target for NDGA is, at the very least, not limited to tubulin. Because plus-end tracking proteins, such as EB1 and dynacitin, play a role in regulating MT dynamics (Carvalho et al., 2003), it is possible that the NDGA, in addition to its direct binding to tubulin, indirectly stabilizes MTs by regulating the function of plus-end tracking proteins.

The action of NDGA on MTs and/or their associated proteins may provide insight into the action of NDGA, not as a lipooxygenase inhibitor or an antioxidant. Lee et al. (2003) reported that NDGA, but not other antioxidants, inhibits transforming growth factor-β activity by blocking the phosphorylation and nuclear translocation of Smad2. This effect of NDGA can be explained by the fact that Smad2 binds to MTs (Dong et al., 2000). Because many transcription factors and protein kinases interact with MTs (Gundersen and Cook, 1999), NDGA may influence transcription and signal transduction by affecting the stability of MTs and/or dynein-dynactin function.

MT-stabilizing reagents such as paclitaxel have been successfully used in the treatment of solid tumors (Bergstrahl and Ting, 2006). The suppression of MT dynamics disrupts the mitotic spindle, halting the cell cycle at the metaphase-anaphase and eventually leading to apoptosis (Yvon et al., 1999). In the presence of NDGA, the mitotic spindle seemed to be disrupted, leading to improper chromosome alignment.

Fig. 5. Effect of NDGA on the distribution of dynein-dynactin and its interacting proteins in mitotic cells. HeLa cells were synchronized by double thymidine block. At 6 h after washing out thymidine, the cells were incubated for 1 h with 0.1% DMSO (Vehicle) or 30 μM NDGA (+NDGA) and then double-stained with the indicated Abs (A) or with ZW10 and propidium iodide (PI) (B). Scale bar, 5 μm. The percentage of cells with abnormal chromosome alignment was scored. Error bars represent the S.E.M. for four experiments.

Fig. 6. Redistribution of TfR by NDGA. A, HeLa cells were incubated for 1 h with 0.1% DMSO (Vehicle) or 30 μM NDGA (+NDGA), fixed with 4% paraformaldehyde at room temperature for 20 min and double-stained with Abs against TfR and a Golgi marker, syntaxin 5 (Syn 5). Scale bar, 5 μm. B, HeLa cells were preincubated in the absence of fetal calf serum for 1 h to remove endogenous Tf, and then 0.1% DMSO (Vehicle) or 30 μM NDGA (+NDGA) was added. After a 1-h incubation, Tf-FITC was added to a final concentration of 25 μg/ml, and the incubation was continued for another 1 h. The cells were stained with an Ab against TfR. C, HeLa cells were incubated with 25 μg/ml Tf-FITC for 1 h to allow the uptake of ligand. The cells were washed and incubated with 0.1% DMSO (Vehicle) or 30 μM NDGA (+NDGA). After a 1-h incubation, the cells fixed and stained with an Ab against TfR. D, HeLa cells were synchronized by double thymidine block. At 6 h after washing out thymidine, the cells were treated for 1 h with 0.1% DMSO (Vehicle) or 30 μM NDGA (+NDGA) and then double-stained with Abs against TfR and α-tubulin.

Scale bar, 5 μm.
not to be substantially disrupted. The different effects of paclitaxel and NDGA on the mitotic spindle are consistent with the observations that NDGA does not affect the radiation of MTs originating from the centrosome in interphase cells, whereas paclitaxel perturbs MT array (Nakamura et al., 2003). The misalignment of chromosomes induced by NDGA might be due to premature removal of spindle checkpoint proteins, such as ZW10, from kinetochores. Although the mechanisms of stabilization of MTs by paclitaxel and NDGA are probably different, the stabilization of MT by NDGA also seems to halt the cell cycle at the metaphase-anaphase. This may explain why NDGA causes apoptosis in different tumor xenografts (Avis et al., 1996; Seufferlein et al., 2002).

In summary, we have disclosed a novel action of NDGA (i.e., stimulation of processes mediated by the MT motor dynein-dynactin complex). The anticancer and other drug activities of NDGA should be investigated in the light of MT-related processes.

References


