Endoreduplication of human smooth muscle cells induced by 2-methoxyestra diol: a role for cyclin-dependent kinase 2

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Endoreduplication of human smooth muscle cells induced by 2-methoxyestradiol: a role for cyclin-dependent kinase 2. Am J Physiol Heart Circ Physiol 292: H1313–H1320, 2007. First published October 20, 2006; doi:10.1152/ajpheart.00867.2006.—Endoreduplication has been suggested to contribute to the development of hypertrophy of smooth muscle cells (SMCs) in hypertension. However, endoreduplication in vascular SMCs and the underlying molecular mechanisms are not clear. Treatment of human SMCs with 10 μM 2-methoxyestradiol (2-ME) for 24 h induces accumulation of cells with ≥4N DNA content, and some polyploid/aneuploid cells actively synthesize their DNA, suggesting the occurrence of endoreduplication. In addition, 2-ME treatment upregulates the expression of cyclin-dependent kinase 2 (Cdk2). The present study was designed to characterize endoreduplication of human SMCs and explore the potential roles of Cdk2 in endoreduplication induced by 2-ME. Treatment with 2-ME (10 μM) for 2–4 days not only caused increases in >4N cells and their reentry into S phase but also induced overduplication of chromosomes. Furthermore, 2-ME increased the kinase activity of Cdk2 and its interaction with cyclin E. Inducible overexpression of dominant-negative Cdk2 in human SMCs inhibited both DNA synthesis of >4N cells and the accumulation of >4N cells induced by 2-ME. We conclude that 2-ME induces endoreduplication of human SMCs and Cdk2 plays an important role in endoreduplication in response to 2-ME.

estrogen metabolite; polyploidization; cell cycle; laser scanning; cytometry; microtubule-interfering agents

ENDOREDUPICATION, A PROCESS OF uncoupling of DNA reduplication from cell division, gives rise to polyploid cells with increased chromosome number to multiples of the normal haploid (6). Endoreduplication has been observed in cells after prolonged exposure to microtubule-interfering agents (MTIs) (2, 18). In response to MTIs, cells can transiently arrest at preanaphase because of activation of the mitotic checkpoint, followed by aberrant exit from mitosis and formation of tetraploidy (4N cells) (2). If the tetraploid G1 checkpoint is not intact, the 4N cells may inappropriately continue to the next cell cycle and enter S phase, resulting in cells with DNA content greater than 4N (>4N cells). It has been suggested that a p53-dependent pathway is involved in the tetraploid checkpoint and regulation of endoreduplication, since the tetraploid cells enter S phase and endoreplicate their DNA in p53-deficient cells (21). Furthermore, in human cancer cell lines, mitosis spindle disruption-induced endoreduplication was prevented by overexpression of p21 or by a Cdk pharmacological inhibitor, suggesting that cyclin-dependent kinase 2 (Cdk2) activity might play a role in endoreduplication (22, 28).

2-Methoxyestradiol (2-ME), an endogenous metabolite of estrogen, has been well documented to have antiproliferative and antiangiogenic effects both in vitro and in vivo. The antiproliferative effects arise mainly from triggering of apoptosis (20, 26). The mechanisms underlying 2-ME actions may involve inhibition of tubulin polymerization (4, 30) and are independent of estrogen receptors (α/β) (19). Furthermore, 2-ME can inhibit tubulin polymerization by interacting at the colchicine site (3, 5). This raises the possibility that 2-ME, like other MTIs, such as colchicine and nocodazole (12, 25), could induce endoreduplication.

Endoreduplication and the formation of polyploid smooth muscle cells (SMCs) have been suggested to contribute to the development of hypertrophy of vascular SMCs in hypertension (23). Recent studies found that, in response to mitotic spindle damage, vascular SMCs derived from prehypertensive rats have a larger proportion of >4N cells than those from normotensive strains and that vascular SMCs derived from hypertensive animals can undergo polyploidization through the cyclin kinase-associated protein (Cks1)-mediated pathway (13). However, endoreduplication in vascular SMCs and the underlying mechanisms are still largely unknown (1). On the basis of the observations from previous studies that treatment with 2-ME (10 μM) for 24 h induced accumulation of 4N cells, caused some polyploid/aneuploid 4N cells to actively synthesize DNA, and upregulated Cdk2 expression (9), the present study was designed to characterize endoreduplication of human SMCs and explore the underlying molecular mechanisms, with a focus on the potential role of Cdk2 in the regulation of SMC endoreduplication in response to 2-ME.

MATERIALS AND METHODS

Materials. Human vascular SMCs derived from aorta were purchased from American Type Culture Collection (Manassas, VA). Kaiglin’s modification of Ham’s F12 (F12K) medium, fetal calf serum, trypsin-EDTA, insulin, transferrin, and selenium were purchased from Invitrogen (Burlington, ON, Canada). Antibodies against cyclin E and Cdk2 were from BD Biosciences (Mississauga, ON, Canada). Anti-p53, anti-phospho-p53 (Ser15), anti-phospho-Rb (Ser397/408), and Rb carboxy-terminal (Rb-C) control protein were purchased from Cell Signaling Technology (Beverly, MA). 2-ME and anti-p27 were from Sigma (Oakville, ON, Canada).

Cell culture. Human SMCs were cultured in F12K medium containing 10% fetal calf serum and supplemented with insulin-selenium-transferrin as recommended by American Type Culture Collection.
After they were seeded in the F12K medium with 10% serum for 48 h, cells were treated with and without 2-ME for selected periods of time, as indicated.

**Plasmid DNA construction and generation of stable cells.** The cDNA encoding a dominant-negative mutant of Cdk2 (Cdk2-dn) with Asp145 to Asn in the kinase domain was removed from the plasmid pCMV-Cdk2-dn (31) (generously provided by Dr. Sander van den Heuvel) by BamHI digestion and cloned into pcDNA4/TO/myc-His A (Invitrogen) at the BamHI site. The sequence of Cdk2-dn was confirmed by the DNA Services Core Facility (University of Calgary). Human SMCs were cotransfected with pcDNA6/TR (Invitrogen) and either pcDNA4/TO/Myc or pcDNA4/TO/Myc-Cdk2-dn at a ratio of 6:1 (wt/wt) using Lipofectamine 2000. Colonies resistant to blasticidin (10 μg/ml) were selected for Tet repressor (TR), and those resistant to both blasticidin and zeocin (200 μg/ml) were dual-selected for TR and Cdk2-dn. The stable cells containing a tetracycline-inducible T-rex system were treated with and without doxycycline (1 μg/ml) for 24 h as described previously (33), followed by screening for their capacity to induce expression of Cdk2-dn by immunoblotting with anti-Cdk2 antibody. Approximately 50% of colonies exhibited inducible expression of Cdk2-dn.

**Fluorescence in situ hybridization.** Chromosome enumeration probe labeled with an Orange fluorochrome (CEP Spectrum Orange probe 16; Vysis, Downers Grove, IL) was used to hybridize to the band 16q11.2, locus D16Z3 of human chromosome 16. Experiments were performed according to the manufacturer’s recommendations (Vysis). Briefly, cells on the coverslips were denatured with 70% formamide-2× SSC at 73°C for 5 min and dehydrated for 1 min sequentially in 70, 85, and 100% ethanol, followed by air drying. Cells were hybridized with the probe for 1 h at 42°C. After the nuclei were washed twice with 0.4× SSC-0.3% Nonidet P-40 and then 2× SSC-0.1% Nonidet P-40 (2 min each), the nuclei were counterstained with DAPI. Cells were examined by fluorescence microscopy and photographed with a charge-coupled device camera with Spot software. The score for fluorescence in situ hybridization analysis was determined with criteria defined by Hopman et al. (14). A minimum of 100 cells was scored for each coverslip.

**Immunofluorescence staining and cell cycle analysis.** Bromodeoxyuridine (BrdU) staining and cell cycle analysis were performed as described previously (10). Briefly, cells grown on coverslips were treated as indicated in the figures and labeled with 10 μM BrdU for 60 min. After fixation in ethanol, cells were permeabilized with 0.25% Triton X-100, and DNA was denatured by 4 N HCl. Cells were then immunostained with anti-BrdU monoclonal antibody and Alexa fluors 488-conjugated secondary antibody. Nuclear DNA was counterstained with propidium iodide (PI) in the presence of RNase A. Cells were either inspected by fluorescence microscopy and photographed with a charge-coupled device camera with Spot software or scanned by laser scanning cytometry (LSC; Compucyte, Cambridge, MA). S phase cells were BrdU positive and are shown in green. LSC detected those cells with high FITC values. Nuclear DNA was stained with PI and is shown in red, and DNA content in each cell was scanned by LSC as PI integral. A minimum of 1,000 cells was scanned on each coverslip. The BrdU incorporation rate (%) is presented as percentage of BrdU-positive cells vs. total scanned cells.

**Western blotting and immunoprecipitation.** SMCs grown in 100-mm culture dishes were lysed after various treatments, followed by protein extraction. Equal amounts of protein from each sample (80 μg) were separated by 11% SDS-PAGE and transferred to nitrocellulose membrane, which was incubated with anti-Cdk2 (1:1,000 dilution), followed by horseradish peroxidase-coupled secondary antibody (1:3,000 dilution). Peroxidase activity was detected with an enhanced chemiluminescence detection kit (Cell Signaling). For immunoprecipitation of cyclin E, p27, and Cdk2 by anti-Cdk2, 200 μg of protein from each sample was incubated with 20 μl of anti-Cdk2 antibody and 20 μl of Sepharose A with gentle rocking at 4°C overnight. The immunoprecipitates were subjected to 11% SDS-PAGE. Anti-cyclin E, anti-p27, and anti-Cdk2 antibodies at 1:1,000 dilutions were used as the primary antibodies to detect the respective proteins.

**In vitro Cdk2 assay.** Cells were treated with and without 2-ME for various times, followed by lysis of cells and extraction of protein as described for Western blotting. Equal amounts of protein (200 μg) from each cell lysate were incubated with 20 μl of anti-Cdk2 antibody and Sepharose A at 4°C overnight. The immune complex was collected by centrifugation at 8,000 g for 30 s, followed by washing once with lysis buffer and twice with kinase buffer (25 mM Tris, pH 7.5, 5 mM β-glycerophosphate, 2 mM DTT, 0.1 mM Na3VO4, and 10 mM MgCl2). The complex was then incubated at 30°C for 30 min with 50 μl of kinase buffer containing 200 μM ATP and 2 μg of Rb-C fusion protein as the substrate. The reaction was terminated by addition of 12.5 μl of 5× SDS sample buffer. Samples were boiled for 5 min. Equal volumes (25 μl) of each supernatant were subjected to 11% SDS-PAGE for Western blot detection with phospho-Rb (Ser807/811) antibody (1:1,000 dilution) and secondary antibody coupled to horse radish peroxidase, as described for Western blotting. Rb-C fusion protein (2 μg) phosphorylated by Cdk in vitro (Cell Signaling) was used as a positive control.

**Statistical analysis.** Results are presented as means ± SE. Statistical analysis was performed with Student’s t-test for unpaired groups or with ANOVA followed by Bonferroni’s correction for comparisons of three or more groups. A P value <0.05 was considered a significant difference.

**RESULTS**

**2-ME induces endoreduplication of human vascular SMCs.** In our previous studies in which we investigated the effects of 2-ME on human SMCs (9), we observed that treatment with 2-ME (10 μM) for 2 days induced accumulation of cells with ≥4N DNA content and that some tetraploid/aneuploid 4N cells continued their DNA synthesis, suggesting that the 2-ME treatment caused endoreduplication of human SMCs. To further characterize the endoreduplication induced by 2-ME, we extended the treatment of SMCs with 2-ME (10 μM) for up to 4 days. It was anticipated that prolonged treatment with 2-ME would generate more cells with DNA content greater than 4N (>4N cells) from 4N cells if endoreduplication occurs. LSC analysis showed that >4N cells and even 8N cells were increased after treatment with 2-ME for 4 days (Fig. 1A). In addition, cells at S phase (indicated by their incorporation of BrdU) were analyzed by LSC. The scattergrams showed that treatment with 2-ME increased BrdU-positive cells with ≥4N DNA content (Fig. 1B). Quantitative data of BrdU-positive cells with ≥4N DNA content from five independent experiments are indicated in Fig. 1B (P < 0.01, n = 5). Insets shown in Fig. 1B are representative nuclear images corresponding to BrdU-positive cells with ≤4N (left) and >4N DNA content (right) detected by the relocation feature of LSC. Note that the BrdU-positive cells with >4N DNA content have enlarged, globular, or irregular nuclei.

To confirm endoreduplication of the chromosomes, cells treated with and without 2-ME were examined by fluorescence in situ hybridization analysis with the use of a human chromosome 16 centromeric probe. Representative micrographs are shown in Fig. 2A. The orange dots represent positive signals produced by the presence of sequences homologous to the probe either on unreplicated chromosomes or on pairs of sister chromatids after reduplication. After treatment with 2-ME for 2–4 days, cells exhibited multiple signals, some displaying up to seven or eight signals for the chromosome copies. Histo-
grams generated by scoring the signals of 100 nuclei from randomly selected areas before and after 2-ME treatment (Fig. 2B) showed that most control cells contained two to four signals, but cells treated with 2-ME showed increased chromosome signals of five to eight copies (n = 5), indicating a single round of endoreduplication.

Treatment with 2-ME increases the kinase activity of Cdk2. Cdk2 is essential for the transition from G1 to S and progression of S phase (16, 27). To determine whether 2-ME induction of endoreduplication is through regulation of Cdk2, cells treated without and with 2-ME for up to 4 days were lysed for Western blot detection of Cdk2. The expression of Cdk2 was increased in response to 2-ME in a time-dependent manner (Fig. 3, A and B). To explore further the involvement of Cdk2 in endoreduplication induced by 2-ME, an in vitro Cdk2 kinase assay was performed. As shown in Fig. 3, C and D, the kinase activity of Cdk2 in cells treated with 2-ME was significantly increased, as represented by its ability to phosphorylate the substrate, Rb-C fusion protein (Fig. 3C). The increase in kinase activity of Cdk2 was also time dependent, consistent with upregulated expression of Cdk2 protein. The increase in Cdk2 expression and its activity in response to 2-ME indicates that Cdk2 may play a role in endoreduplication induced by 2-ME.

Treatment with 2-ME increases the interaction of Cdk2 with cyclin E and decreases the association of Cdk2 with p27. It is well known that activated Cdk2 can form a complex with cyclin A or cyclin E during the transition from G1 to S and progression of S phase. To evaluate further the role of Cdk2,
we performed immunoprecipitation assays to detect the interaction of Cdk2 with its cyclin partners, cyclins A and E. We previously reported that the expression level of cyclin E was not changed in response to 2-ME (9). However, treatment with 2-ME, which increased expression and activity of Cdk2, resulted in a significant increase of cyclin E in Cdk2 pull-down assays (Fig. 4, A and B). In contrast, the level of immunoprecipitated cyclin A was low and no significant change was observed in cells with and without 2-ME treatment (data not shown). These results suggest that 2-ME treatment promotes the interaction between Cdk2 and cyclin E. To study further the regulation of Cdk2 activity in human SMCs in response to 2-ME, we also examined the interaction of Cdk2 with p27 and p21, Cdk2 inhibitors. Immunoprecipitation assays revealed that the level of p27, but not p21 (data not shown), was significantly reduced in the cells treated with 2-ME compared with control cells (Fig. 4, A and C), suggesting a decrease in the association of Cdk2 and p27.

Overexpression of Cdk2-dn reduces the accumulation of >4N cells and their BrdU incorporation in response to 2-ME treatment. Our data suggest the involvement of Cdk2 activation in 2-ME-induced cellular responses. To investigate whether Cdk2 plays a critical role in 2-ME-induced endoreduplication, we generated stable cells harboring an inducible system to overexpress Cdk2-dn as represented by the colony D4 (Cdk2-dn-D4) (Fig. 5A). Stable cells harboring the TR system and vector DNA were used as controls. The inhibition of Cdk2 activity by overexpression of Cdk2-dn was confirmed with the in vitro kinase assay (Fig. 5B). LSC analysis showed that cells from five randomly selected colonies had cell cycle profiles in response to 2-ME similar to those from Cdk2-dn-D4 (Fig. 5, C–F). In control cells without induction of Cdk2-dn, treatment with 2-ME for 48 h caused remarkable accumulation of 4N cells and >4N cells. In cells with overexpression of Cdk2-dn induced by doxycycline, the accumulation of >4N cells in response to 2-ME was significantly reduced compared with cells without Cdk2-dn induction (19 ± 2% vs. 29 ± 3%, n = 4; P < 0.01) (Fig. 5, F and D), although the number of 4N cells induced by 2-ME was comparable to that in the control cells. Furthermore, in the absence of 2-ME, only a few BrdU-positive >4N cells were observed with or without doxycycline induction (Fig. 5, C and E). However, in the presence of 2-ME, the BrdU-positive >4N cells were significantly decreased in cells with overexpression of Cdk2-dn compared with cells without Cdk2-dn induction (11 ± 2% vs. 18 ± 3%, n = 4; P < 0.01) (Fig. 5, J and H). Therefore, the inducible expression of Cdk2-dn markedly inhibited both the accumulation of >4N cells and DNA synthesis of >4N cells.

Fig. 2. 2-ME induces overduplication of chromosomes. A: fluorescence in situ hybridization (FISH) for chromosome 16 (orange). The nuclei were counterstained with DAPI (blue). Note that, in the presence of 2-ME (day 2 and day 4), some cells with enlarged, polyploid nuclei contain 7 or 8 copies of 16q11.2 signals. B: summarized data of FISH analysis for the centromeric 16q11.2 probe signals in cells treated with or without 2-ME for the times indicated. A minimum of 100 cells was scored in each coverslip (n = 3).
To evaluate whether p53 activation was involved in Cdk2-mediated endoreduplication, we examined p53 expression and its phosphorylation status in stable cells harboring inducible Cdk2-dn with and without the presence of 2-ME. We found that, with or without 2-ME treatment, there was no significant change in the phosphorylation of p53 (Ser15) or p53 expression level between the control cells and the cells with induction of Cdk2-dn (data not shown).

Fig. 3. Treatment with 2-ME upregulates the expression and kinase activity of cyclin-dependent kinase 2 (Cdk2). A: cells treated with and without 2-ME for the times indicated were lysed. The expression of Cdk2 was detected by immunoblotting with Cdk2 antibody from 80 μg of lysate protein in each sample. Actin was used as a loading control. B: summarized data of the abundance of Cdk2 as measured by densitometry (relative to actin loading control). *Significant difference of Cdk2 levels in cells (n = 4) treated with 2-ME when compared with control cells without 2-ME treatment (day 0) (P < 0.01). C: kinase activity of Cdk2 determined by an in vitro kinase activity assay. After immunoprecipitation of Cdk2 from 200 μg of protein of each lysed sample, 2 μg of Rb carboxy-terminal (Rb-C) fusion protein as the substrate and 200 μM of ATP were added to initiate the reaction. Cdk2 activity, represented by phosphorylation of Rb, was detected with anti-phospho-Rb (Ser807/811) by immunoblotting. Phosphorylated Rb-C protein (2 μg) was used as the positive control. D: summarized results of Cdk2 kinase activities determined by densitometric measurement of the abundance of phosphorylated Rb from 3 independent experiments. *Significant difference compared with untreated cells (n = 3; day 0 control) (P < 0.01).

Fig. 4. Interaction of Cdk2 with cyclin E and p27 in response to 2-ME treatment. A: cell lysate protein (200 μg) from cells treated with and without 2-ME up to 4 days was immunoprecipitated with anti-Cdk2, followed by immunoblotting with anti-cyclin E, anti-p27, and anti-Cdk2 antibodies, respectively. B: quantification of densitometric measurements of cyclin E immunoprecipitated by Cdk2. *Significant changes of cyclin E level compared with the day 0 control (P < 0.01, n = 3). C: quantification of densitometric measurements of p27 immunoprecipitated by Cdk2. *Significant differences compared with the day 0 control (P < 0.01, n = 3).
DISCUSSION

Endoreduplication has been observed in cells treated with MTIs, such as colchicine and nocodazole (12, 25). The present study demonstrated that 2-ME treatment induces the accumulation of $>$4N cells, increases $>$4N cells reentry into S phase, and causes overduplication of chromosomes in tetraploid and/or aneuploid cells. These results confirm that 2-ME induces endoreduplication of human SMCs and support the concept that MTIs, which damage the mitotic checkpoints, can switch on a new replication cycle without anaphase chromosome segregation. The question is how mitotic spindle damage initiates a new round of DNA synthesis in 4N cells. It has been shown that DNA damage can cause endoreduplication (15). Our studies demonstrate that mitotic spindle disruption causes the formation of polyploid/aneuploid cells and endoreduplication. Therefore, it is possible that polyploid/aneuploid cells, induced by microtubule disruption due to aberrant chromosome segregation and cytokinesis, may contain abnormal amounts of and/or damaged DNA, which in turn results in the occurrence of endoreduplication. In addition, our results are in agreement with
the observations in hypertensive animal-derived SMCs, which undergo polyploidization after mitotic spindle damage (13). However, polyploidization of SMCs derived from hypertensive animals was mediated by Cks1 through promotion of cyclin B degradation (13). We did not detect significant changes of cyclin B levels with and without 2-ME treatment (9), implying that a different pathway(s) or mechanism(s) is involved in endoreduplication and polyploidization of SMCs. Although the possible contribution of endoreduplication and polyploidization of vascular SMCs to the pathogenesis of hypertension in human subjects remains to be determined, the present study regarding 2-ME effects on the induction of endoreduplication and polyploidization in human SMCs provides enhanced understanding of 2-ME actions.

In human breast cancer and colon cancer cell lines, endoreduplication induced by MTIs is associated with deregulated cyclin E/Cdk2 activity (22, 28). However, evidence for a link between Cdk2 and endoreduplication was lacking. Our results show that the kinase activity of Cdk2, concurrent with the increase in expression of the protein, was upregulated in response to 2-ME. Importantly, overexpression of Cdk2-dn through an inducible system not only inhibited the accumulation of >4N cells but also reduced >4N cell reentry into S phase induced by 2-ME. These results provide direct evidence that Cdk2 plays an important role in the 2-ME-induced endoreduplication in human vascular SMCs. Although the increase in Cdk2 activity by treatment with 2-ME was prevented by overexpression of Cdk2-dn, endoreduplication induced by 2-ME was not completely inhibited. It is possible that a Cdk2-independent mechanism is involved. However, we cannot rule out the possibility that the basal Cdk2 activity, which is below detectable levels of the kinase assay, still has regulatory effects on cell cycle, as the case for the control cells. Furthermore, our results demonstrate that treatment with 2-ME reduces the association of Cdk2 with p27 and increases the interaction of Cdk2 with cyclin E. Therefore, it is conceivable that microtubule disruption causes activation of Cdk2 through dissociation of Cdk2 from its inhibitor, p27, and association with its cyclin partner, cyclin E, leading to S phase entry. In addition, it was shown that disruption of mitotic spindles causes endoreduplication in p53-negative, but not p53-positive, mouse embryonic fibroblasts (18), suggesting that functional p53 is essential to prevent endoreduplication. In our study, we did not detect a significant change in either total p53 levels or phosphorylation of p53 (Ser15) between cells with or without Cdk2-inducible overexpression in the presence or absence of 2-ME treatment. This suggests that phosphorylation of p53 at Ser15 is not involved in the endoreduplication induced by 2-ME. However, we could not rule out the possibilities that phosphorylation of p53 at residue(s) other than Ser15 treatment is involved. It is also possible that in response to 2-ME human SMCs may lack functional p53, resulting in the occurrence of endoreduplication.

2-ME is well known for its effects of antiangiogenesis and antitumor growth (8, 24) and has entered phase II clinical trials (29). Plasma concentrations of 2-ME are in the picomolar ranges under normal physiological conditions and increase 1,000-fold during late pregnancy (17). In the clinical trial participants, the plasma concentrations of 2-ME can reach 24 ng/ml after uptake of a single dose of 2-ME (1,200 mg) (29). Additionally, vascular SMCs can metabolize estradiol to produce 2-ME (32). It is possible that lipophilic 2-ME has higher concentrations in some regions of the blood vessel wall than in the plasma. Under certain pathological conditions, such as atherosclerosis and restenosis, proliferative vascular SMCs might have a potential to undergo an aberrant cell cycle in response to stimuli. Our study demonstrates that short-term exposure to 2-ME (12–24 h) causes disruption of the mitotic spindle, mitotic arrest, and apoptosis, whereas long-term exposure to 2-ME (2–4 days) induces endoreduplication. The effective concentrations of 2-ME (1–10 μM) used in our study are in agreement with the majority of published work that have used cancer cell lines in in vitro studies. Although our in vitro studies do not reflect the in vivo situation per se, these observations raise the question of whether long-term treatment with 2-ME could create the potential risk of genomic instability, since endoreduplication can generate more polyploidy/aneuploidy, which is believed to contribute to the development of cancer (7, 11). Our study also demonstrates that Cdk2 plays an important role in the endoreduplication induced by 2-ME. Therefore, it is reasonable to predict that inhibition of Cdk2 may provide an alternative way to reduce the risk of polyploidy/aneuploidy during treatment with 2-ME.

In summary, our results demonstrated that 2-ME induces endoreduplication of human vascular SMCs and that activation of Cdk2 plays an important role in endoreduplication in response to 2-ME. The finding that 2-ME induces endoreduplication of human cells raises concerns as to whether clinical application of 2-ME may risk induction of polyploidy/aneuploidy and whether the occurrence of polyploidy/aneuploidy could contribute to the pathogenesis of secondary cancers.

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