Increased D-type Cyclin Expression Together with Decreased cdc2 Activity Confers Megakaryocytic Differentiation of a Human Thrombopoietin-dependent Hematopoietic Cell Line*

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At the late phase of megakaryocytopoiesis, megakaryocytes undergo endomitosis, which is characterized by DNA replication without cell division. Although a number of cell cycle regulatory molecules have been identified, the precise roles of these molecules in megakaryocytic endomitosis are largely unknown. In a human interleukin-3-dependent cell line transfected with the thrombopoietin (TPO) receptor c-mpl (F-36P-mpl), either treatment with TPO or the overexpression of activated ras (Ha-RasG12V) induced megakaryocytic maturation with polyploid formation. We found that TPO stimulation or Ha-RasG12V expression led to up-regulation of cyclin D1, cyclin D2, and cyclin D3 expression. In addition, expression levels of cyclin A and cyclin B were reduced during the total course of both TPO- and Ha-RasG12V-induced megakaryocytic differentiation, thereby leading to decreased cdc2 kinase activity. Neither the induced expression of cyclin D1, cyclin D2, or cyclin D3 nor the expression of a dominant negative form of cdc2 alone could induce megakaryocytic differentiation of F-36P-mpl cells. In contrast, overexpression of dominant negative cdc2 together with cyclin D1, cyclin D2, or cyclin D3 facilitated megakaryocytic differentiation in the absence of TPO. These results suggest that both D-type cyclin expression and decreased cdc2 kinase activity may participate in megakaryocytic differentiation.

Cell growth and differentiation is tightly regulated by a series of cell cycle regulatory molecules such as cyclins, cyclin-dependent kinases (cdks), and Cdk inhibitors (for a review, see Ref. 1). A number of previous studies have revealed that cell cycle arrest is closely related with and in some cases sufficient for inducing differentiation. For example, expression of Cdk inhibitors such as p21waf1, p27kip1, and p18ink4c was induced or up-regulated in muscle cells, keratinocytes, and B lymphoblastoid cells during terminal differentiation (2–4). Furthermore, ectopic overexpression of p21waf1, p18ink4c, and p19ink4d was shown to induce terminal differentiation of human megakaryoblastic leukemia cells lines CMK and UT7, murine myeloblastic cell line 32D c13, and B-lymphoblastoid cells, respectively (4–7). When this coordination was deregulated by the inappropriate overexpression of positive cell cycle regulators such as cyclin, cdk4, or E2F, the cells underwent differentiation arrest or apoptosis (8–11).

Megakaryocytopoiesis is performed through a series of complex processes that involve proliferation of committed precursor cells and subsequent differentiation of their progeny leading to cytoplasmic maturation and platelet fragmentation. During the late phase of megakaryocytopoiesis, megakaryocytes are known to undergo endomitosis (also called endoreplication), which is characterized by DNA replication without concomitant cell division. In hematopoietic cells, endomitosis is a phenomenon unique to megakaryocytic lineage cells, while it is also observed in other cell types, including cells of liver, urinary bladder epithelium, trophoblast and salivary gland (12, 13). Although a number of investigators have investigated the mechanisms of megakaryocytic endomitosis, the results of these studies were contradictory: some studies demonstrated that endomitosis was accompanied by low cdc2 activities due to the down-regulation of CDC25C phosphatase or the decreased expression of cyclin B1 (14–16), but a different study showed that cdc2 activity was retained in the endomitotic polyploid megakaryocytes (17). Thus, the precise mechanism underlying megakaryocytic endomitosis remains largely unknown.

Thrombopoietin (TPO) was cloned as a ligand for the c-mpl proto-oncogene. It is a member of hematopoietin receptor superfamily with high sequence similarity to the receptors for erythropoietin and granulocyte colony-stimulating factor. The c-Mpl is expressed in hematopoietic tissues, particularly in CD34+ hematopoietic progenitor cells, megakaryocytes, and platelets, while its ligand TPO is primarily produced in the liver, kidney, and smooth muscle, with lesser amounts present in the spleen and bone marrow (for a review, see Ref. 18). A number of in vitro experiments have shown that TPO acts at various stages of megakaryocytic differentiation, including proliferation of megakaryocytic progenitor cells, endomitosis, and...
cytoplasmic maturation (19–21). In addition, it has been shown that daily infusion of TPO into mice or nonhuman primates induces a marked increase in the number of platelets, megakaryocytes, and megakaryocytic progenitor cells (22, 23). Furthermore, the c-mpl- or TPO-deficient mice generated by gene targeting were reported to reveal a striking decrease in the number of platelets and megakaryocytic progenitor cells (24, 25). These results suggested that the TPO/c-Mpl system is a physiologic regulator of megakaryopoiesis and platelet production.

The binding of TPO to c-Mpl activates a variety of signaling molecules such as the Janus family of protein tyrosine kinases (JAK), signal transducers and activators of transcription (STAT) and Ras, and recent studies have provided some insight into functional domains of c-Mpl and TPO-activated signaling molecules associated with cell growth and differentiation (26–30). By using a c-mpl-transfected, interleukin-3 (IL-3)-dependent human erythroleukaemia cell line, F-36P-mpl, that can differentiate at a high rate into mature megakaryocytes in response to TPO, we have previously shown that prolonged Ras activation was crucial for TPO-induced megakaryocytic differentiation (30). Furthermore, megakaryocytic differentiation of F-36P-mpl cells could be induced by the prolonged (greater than 24 h) expression of activated Ras (Ha-RasG12V) (30). These results suggested that TPO-activated Ras signaling might be involved in the regulation of megakaryocytic endomitosis. However, the molecular mechanisms of TPO- or activated Ras-mediated endocytosis remain to be determined.

In this study, we have investigated the effects of TPO and Ha-RasG12V on the expression and function of cell cycle regulators and have found that TPO- and Ha-RasG12V-induced megakaryocytic differentiation of F-36P-mpl cells was accompanied with the sustained expression of D-type cyclins and decreased cdc2 kinase activity. Furthermore, we have demonstrated that overexpression of each D-type cyclin along with a dominant negative (dn) form of cdc2 was sufficient for inducing megakaryocytic differentiation of F-36P-mpl cells. Thus, we provide evidence that increased cyclin D expression together with decreased cdc2 activity may contribute to megakaryocytic endomitosis.

**EXPERIMENTAL PROCEDURES**

**Reagents and Antibodies**—Highly purified recombinant human (rh) TPO and rhIL-3 were provided by Kirin Brewery Company Ltd. (Tokyo, Japan). AP2 (anti-human GP IIb-IIIa complex) monoclonal antibody was generously provided by Dr. T. Kunicki (Scripps Research Institute, La Jolla, CA) (31). Murine anti-cyclin D1, cyclin D2, cyclin D3, and cdc2 monoclonal antibodies were purchased from Pharmingen (San Diego, CA). Murine anti-cdk4 and anti-cdc2 Abs were purchased from Transduction Laboratories (Lexington, KY). Rabbit anti-cdk6 Ab and glutathione S-transferase-Rb (GST-Rb) protein were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA).

**Cell Lines and Cultures**—F-36P, a human IL-3-dependent erythrocytopenia cell line established by Chiba et al. (32) was obtained from Riken Cell Bank (Takata, Japan). F-36P-mpl, F-36P transfected with c-mpl, and F-36P-H-rasG12V, F-36P transfected with a Lac-inducible expression vector of Ha-RasG12V, were described previously (30). These cells were cultured in RPMI 1640 (Nakatani, Tokyo, Japan) supplemented with 10% fetal calf serum (Flow, North Ryde, Australia) in the presence of 10 μM β-2M (Flow, North Ryde, Australia) in the presence of 10 ng/ml rhIL-3 at 37 °C.

**Plasmid Construct and cDNAs**—Human cyclin D1 cDNAs was generously provided by Dr. A. Arnold (Massachusetts General Hospital, Boston, MA). Human cyclin D2 and D3 cDNAs were supplied by Dr. G. Peters (Imperial Cancer Research Fund, London, United Kingdom). A dn form of cdc2 was a gift from Dr. Ed Harlow (Massachusetts General Hospital, Boston, MA). These cDNAs were subcloned into NotI site of pRSV2 (Stratagene, La Jolla, CA) replacing the chloramphenicol acetyltransferase gene by using NotI linkers. Other cDNAs were kindly provided from the investigators as follows: human p21(Cip1) from Dr. A. Noda (Meiji Institute of Health Science, Odawara, Japan); human p27(Kip1) cyclin A, cyclin B, and cdk2, and cdk4 from Dr. H. Ykiyama (University of Illinois, Cancer Center, Chicago, IL); human p16(Ink4a) from Dr. T. Nobori (University of California, San Diego, CA); human p15(Ink4b) from Dr. K. Kataoka (Institute of Medical Science Tokyo University, Tokyo, Japan); murine p18(Ink4c) from Dr. C. Sherr (St. Jude Children’s Research Hospital, Memphis, TN), respectively.

**Lac-inducible System**—To obtain a target cDNA, we used a Lac-switchTM inducible expression system (Stratagene). In short, F-36P-mpl cells, that are already transfected with an expression vector of Lac repressor (Lac-R), pCMV-LacI, were further transfected with pOPRSV1 each containing cyclin D1, cyclin D2, cyclin D3, and dn-cdc2. After culture with G418 (Sigma) at a concentration of 1.5 mg/ml G418-resistant cells were cloned and induction levels of the target protein were measured before and after treatment with 0.5 mM IPTG by western blot analyses. The expression vector pOPRSV1 contains the RSV promoter linked to the Escherichia coli lactose operon, and expression of a target cDNA is suppressed by the Lac-R through the lactose operon. When IPTG was added to culture medium, Lac-R was released from lactose operon and transcription of the target cDNA was initiated.

**Flow Cytometry**—The surface expression of GP IIb-IIIa was examined with AP2 monoclonal antibody by the indirect immunofluorescent method on FACSort (Beckon Dickinson, Oxnard, CA) (33). The DNA content of cultured cells was quantitated by staining with propidium iodide and analyzed on FACSort as described previously (5).

**Northern Blot Analysis**—The isolation of total cellular RNA and the method for Northern blot were described previously (34). Changes in Morphology and DNA Content during TPO- and Ha-RasG12V-induced Megakaryocytic Differentiation—In a previous study, we have reported that megakaryocytic differentiation of F-36P-mpl cells was induced by 5-day culture with rhTPO, and that of F-36P-H-rasG12V cells by 5-day treatment with IPTG causing induced expression of activated Ras (Ha-RasG12V) (30). In the present study, we have examined changes in morphology of F-36P-mpl and F-36P-H-rasG12V cells during the rhTPO- and IPTG (Ha-RasG12V)-induced megakaryocytic differentiation. As shown in Fig. 1, both F-36P-mpl and F-36P-H-rasG12V cells were drastically composed of undifferentiated megakaryoblastic cells before culture with rhTPO and IPTG. No significant changes in morphology were observed in both cultures until 24 h. However, considerable proportions of F-36P-mpl and F-36P-H-rasG12V cells revealed morphological changes indicative of megakaryocytic maturation after the culture with rhTPO and IPTG at 72 h (about 15% of the cultured cells in both cultures) and at 96 h (about 40% of the cultured cells in both cultures), respectively.
these changes were more apparent in cytospin preparations after 120 h culture with rhTPO and IPTG, in both of which about 60% of the cultured cells underwent megakaryocytic differentiation. It was of interest to note that most of megakaryocytes developed after induction of Ha-RasG12V by IPTG possessed one giant nucleus, but not multilobular nuclei that were observed after the culture with rhTPO. In agreement with the morphological data, DNA content analysis revealed that both rhTPO and IPTG treatment led to polyploid formation of F-36P-mpl and F-36P-H-rasG12V cells at 120-h after the initiation of the cultures, respectively: at 120 h, rhTPO, 2N 40.5%, 4N 37.0%, 8N 15.3%, 16N 7.2%; Ha-RasG12V, 2N 35.0%, 4N 31.2%, 8N 14.0%, 16N 13.4%, 32N 6.4% (Table I).

Changes in Expression of Cell Cycle Regulating Molecules during rhTPO- and Ha-RasG12V-induced Megakaryocytic Differentiation—In an effort to clarify the mechanism of the rhTPO- and Ha-RasG12V-induced megakaryocytic differentiation, changes in expression of cell cycle regulating molecules including cyclins,Cdks, and Cdks inhibitors were examined by Northern blot analysis. F-36P-mpl and F-36P-H-rasG12V cells were deprived of rhIL-3 for 48 h, and then cultured in the absence of rhIL-3 with rhTPO and IPTG for 5 days, respectively. Expression of cyclin D1 and cyclin D2 mRNA was found to be induced by rhTPO as early as 4 h in F-36P-mpl cells; cyclin D1 expression retained at relatively high levels until 120 h, while cyclin D2 expression decreased at 24 h (Fig. 2). Also, expression of cyclin D1 and cyclin D2 in F-36P-H-rasG12V cells was induced by Ha-RasG12V and sustained at high levels until 120 h. In both culture conditions, cyclin D3 expression was gradually up-regulated for up to 120 h. Expression of cyclin A and cyclin B was transiently induced by rhTPO, reached a peak at 24 h, and then decreased after 48 h. Similarly, expression of cyclin A and cyclin B decreased to an undetectable level after 72 h in F-36P-H-rasG12V cells. Expression of p21waf1 mRNA was not detected during the culture with rhTPO, while its induction was observed at the late phase of Ha-RasG12V-induced differentiation. By contrast, expression of p16ink4a or p15ink4b mRNA was not observed throughout the cultures. In addition, expression of p27kip1, p18ink4c, cdk2, cdk4, and p34cdc2 did not change significantly in both cultures during the test period (Fig. 2). Consistent with the data on Northern blot analyses, Western blot analyses demonstrated that expression of cyclin D1, cyclin D2, and cyclin D3 proteins was induced by rhTPO or Ha-RasG12V with kinetics similar to those observed in the Northern blot (Fig. 3A). Also, expression of cyclin A and cyclin B was found to be reduced after 72 h in both cultures (Fig. 3A). Since cyclin A and cyclin B have been reported to complex with cdc2 and to regulate its activity (for a review, see Ref. 35), we examined changes in cdc2 activity with an immune complex kinase assay by using histone H1 as a substrate. After 48 h starvation of rhIL-3, F36P-mpl and F36P-H-rasG12V cells were cultured in the absence of rhTPO or IPTG for the times indicated, respectively, and subjected to an in vitro kinase assay. During the culture with rhTPO, cdc2 activity was found to transiently increase at 24 h, and to decline thereafter (Fig. 3B). Also, cdc2 activity was gradually down-regulated by Ha-RasG12V (Fig. 3B).

Changes in cdk4 and cdk6 Activities during rhTPO- and Ha-RasG12V-induced Megakaryocytic Differentiation—To further define the roles of the increased D-type cyclins during TPO- and Ha-RasG12V-induced endomitosis, we next investigated changes in cdk4 and cdk6 activities during the both
cultures. After 48 h of IL-3-deprivation, F-36P- mpl and F-36P- H-rasG12V cells were cultured with rhTPO and IPTG for the time indicated, respectively. As shown in Fig. 4A, Western blot analysis on the whole cell lysates demonstrated that expression levels of cdk4 and cdk6 proteins did not show an apparent change during the test period in both cultures. Next, cdk4 and cdk6 were immunoprecipitated from total cellular lysates, and subjected to an immune complex kinase assay by using glutathione S-transferase-Rb as a substrate. Although a transient increase in cdk4 and cdk6 activities was induced by TPO and Ha-RasG12V at 24–48 h (in a proliferative process), their activities were found to decrease slightly during the endomitotic process from 72 to 120 h, despite the up-regulation of cyclin D expression in both cultures (Fig. 4B). However, their activities were still retained at easily detectable levels in both cultures.

Effects of Overexpression of D-type Cyclins and/or dn-cdc2 on Megakaryocytic Differentiation—As rhTPO- and Ha-RasG12V- induced megakaryocytic differentiation was accompanied by the sustained expression of D-type cyclins and decreased cdc2 activity, we prepared stable clones of F-36P- mpl expressing the indicated cyclin or a dominant negative version of cdc2. The cells of each clone were cultured in the presence or absence of rhIL-3 (10 ng/ml) with or without IPTG (0.5 mM) for 5 days. In the absence of IL-3, the induced overexpression of cyclin D1, cyclin D2, or cyclin D3 by IPTG treatment resulted in a significantly increased proportion of apoptotic cells, as noted by the increased subdiploid fractions (cyclin D1, 48.2 versus 14.1%; cyclin D2, 37.3 versus 15.2%; cyclin D3, 44.5 versus 23.6%) (Fig. 5B, Table I). In contrast, when rhIL-3 was added to the culture medium, overexpression of cyclin D1, cyclin D2, or cyclin D3 resulted in the increased fractions of S or G2/M phase cells, but did not induce apoptosis; % of the cells in S or G2/M phase with and without IPTG were as follows: cyclin D1, 68.2 versus 48.2%; cyclin D2, 53.6 versus 43.5%; cyclin D3, 56.7 versus...
demonstrated the importance of both cyclin Ds and cdc2 in regulating molecules located downstream of Ras signaling, and study, we investigated the expression and function of cell cycle megakaryocytes in response to phorbol esters (36–38). In this blastic leukemia cells, both of which gave rise to mature differentiation of K562 erythroleukemia cells and CMK megakaryocytes in response to phorbol esters (36–38).

42.3% (Fig. 5B, Table II). Overexpression of dn-cdc2 did not significantly affect cell growth in the absence of rhIL-3, whereas the apoptotic fraction was slightly reduced from 25 to 11% by IPTG treatment. In contrast, when dn-cdc2 was inducibly expressed in the presence of rhIL-3, only a small proportion of the cells (about 2%) was found to undergo megakaryocytic differentiation by morphological analysis (data not shown). This result was consistent with that of DNA content analysis which revealed an appearance of a small 8N fraction (Fig. 5B). However, none of these molecules alone was able to efficiently induce megakaryocytic differentiation. Therefore, we next examined the combinational effects of these molecules on megakaryocytic differentiation. Three clones that could inducibly express dn-cdc2 in combination with cyclin D1, cyclin D2, or cyclin D3 were established and subjected to flow cytometric and morphological analyses. As shown in Fig. 6A, polyploid formation was observed after 5-day culture with IPTG in the presence of rhIL-3 in all of these clones. Because surface phenotypic maturation and natural maturation (polyploidization) were shown to be executed independently, we examined whether surface maturation was observed in these polyploid megakaryocytes that developed after induction of D-type cyclin and dn-cdc2. As shown in Fig. 6B, surface expression levels of GP IIb-IIIa were up-regulated after 5-day culture with IPTG in these clones. In accord with the results of flow cytometric analysis, cytospin preparations after IPTG treatment for 5 days revealed the changes in morphology indicative of megakaryocytic maturation in all of the clones (Fig. 7). However, most of the megakaryocytes that developed after the induction of dn-cdc2 in combination with cyclin D1, cyclin D2, or cyclin D3 showed one giant nucleus similar to that seen in the case of Ha-RasG12V. Similar megakaryocytic maturation was observed after the treatment with IPTG even in the absence of rhIL-3 (data not shown). These results suggested that the sustained expression of each D-type cyclin together with the decreased activity of cdc2 was sufficient for inducing megakaryocytic differentiation.

### DISCUSSION

The interaction of TPO with c-mpl results in activation of multiple signal transduction pathways, including those of JAK-STAT and Ras-mitogen-activated protein kinase. By using dn-STATs, dn-Ras, and activated forms of STAT5 and Ras (Ha-RasG12V), we have previously shown that prolonged Ras activation is required for TPO-induced megakaryocytic differentiation of F-36P mpl cells (30). It was also reported that an active form of extracellular signal-regulated kinase mitogen-activated protein kinase could induce megakaryocytic differentiation of K562 erythroleukemia cells and CMK megakaryoblastic leukemia cells, both of which gave rise to mature megakaryocytes in response to phorbol esters (36–38). In this study, we investigated the expression and function of cell cycle regulating molecules located downstream of Ras signaling, and demonstrated the importance of both cyclin Ds and cdc2 in megakaryocytic endomitosis.

Although it was postulated that endomitosis was due to skipping of mitosis after each round of DNA replication, recent studies suggested that, during TPO-induced polyploidization, human or murine megakaryocytes may enter mitosis and progress through normal prophase, prometaphase, metaphase, and up to anaphase A, but not to anaphase B, telophase, or cytokinesis (17, 39). As for the roles of cell cycle regulatory molecules in endomitosis, Garcia and Cales (16) showed that cdc2 kinase activity was severely decreased due to down-regulation of cdc25C phosphatase during phorbol ester-induced megakaryocytic differentiation of HEL and Meg-01 cells. Zhang et al. (14, 15) also reported that cdc2 kinase activity was reduced during endomitosis due to ubiquitin-dependent degradation of cyclin B in normal rat megakaryocytes as well as in the megakaryocytic cell lines, MegT and Y10/L8057. However, a recent study reported that cyclin B1 and cdc2 activities could be detected in human endomitotic polyploid megakaryocytes (17). In the present study, we found that cdc2 kinase activity was down-regulated due to the decreased expression of cyclin A and cyclin B during the course of both TPO- and Ha-RasG12V-induced megakaryocytic differentiation, and that dn-cdc2 in combination with a D-type cyclin was capable of inducing polyploid formation in F-36P mpl cells. These results suggested that endomitosis may be performed efficiently under the condition where cdc2 kinase activity was down-regulated.

Because of requirement of DNA synthesis in endomitosis, D-type cyclins that are critically important for cell cycle (G1/S) progression (for a review, see Ref. 1) have been supposed to participate in polyploid formation of megakaryocytes. Wang et al. (40) reported that, when antisense oligonucleotides designed to suppress cyclin D3 expression was added to a primary culture of murine bone marrow cells, development of megakaryocytes was significantly suppressed, while cyclin D1 or cyclin D2 antisense oligonucleotides had little effect. In addition, Zimmet et al. (41) reported that cyclin D3 was expressed at significant levels in polyploid megakaryocytes and

| Type of cyclin | IL-3 (−); apoptotic cell (%) | IL-3 (+); cells in S or G2/M phase (%) |
|---------------|------------------------------|---------------------------------------|
| Cyclin D1     | 14.1                         | 48.2                                  |
| Cyclin D2     | 15.2                         | 37.3                                  |
| Cyclin D3     | 23.6                         | 44.5                                  |
was up-regulated following the *in vivo* exposure to TPO (40). Furthermore, they demonstrated that bone marrow megakaryocytes obtained from transgenic mice, in which cyclin D3 was overexpressed exclusively in the megakaryocytic lineage, showed a significant increase in endomitosis (41). These lines of evidence suggested that cyclin D3 may be a key regulator of DNA replication in endomitosis. By using a refined model of megakaryocytic differentiation, we found that either TPO stimulation or Ha-RasG12V expression could up-regulate the expression of cyclin D3 and cyclin D2 in addition to cyclin D3, and that, when cdc2 activity was suppressed, each of cyclin D1, cyclin D2, and cyclin D3 expression was able to induce megakaryocytic differentiation with a similar efficiency. These findings raised the possibility that functional roles of D-type cyclins may be redundant, and that each D-type cyclin could participate in megakaryocytic endomitosis. However, the up-regulated expression of cyclin D1 and cyclin D2 was observed earlier than that of cyclin D3, and was detectable at mRNA and protein levels prior to the development of polyploid megakaryocytes. It is therefore possible that cyclin D1 or cyclin D2 may be involved in TPO-induced proliferation rather than megakaryocytic differentiation, whereas cyclin D3 may participate in megakaryocytic endomitosis. This possibility is partially supported by our recent finding that cytokines such as TPO appear to regulate cell growth through transcriptional regulation of cyclin D1 by STAT5 and Ras signaling (42). Additional works such as those on cyclin D3 targeted mice are required to elucidate the direct role of cyclin D3 in megakaryocytic endomitosis. Despite the accompanied expression of D-type cyclins, we did not detect distinct elevation of cdk4 and cdk6 activities during TPO- or Ha-RasG12V-induced endomitosis, whereas easily detectable levels of their activities are observed. Therefore, it was speculated that intense cyclin D-dependent kinase activities might not be required for DNA replication during megakaryocytic endomitosis as compared with their necessity in proliferative conditions.

In a previous study, Kikuchi et al. (6) reported that surface phenotype maturation and polyploidization were performed independently. However, in this study, surface expression levels of GP Ila-IIla were up-regulated in the megakaryocytes that developed after induction of dn-cdc2 and D-type cyclin, suggesting that nuclear maturation may be spontaneously accompanied by phenotypic maturation. In addition to megakaryocytic differentiation, macrophage differentiation evoked by cell cycle regulatory molecules such as p21 and p19 was reported to be accompanied by surface phenotypic maturation (7, 43). These results including ours suggested artificially induced nuclear maturation could be linked with surface phenotypic maturation. As in the case of Ha-RasG12V, most of differentiating megakaryocytes that developed in response to dn-cdc2 and D-type cyclin possessed only one giant nucleus but not multinucleated nuclei that were observed in F-36P mpl cells after culture with TPO. These results suggested the presence of an additional mechanism(s) that regulates the maturation of polyploid megakaryocytes. Further studies on this TPO/F36-P mpl system would undoubtedly provide greater insights into molecular controls of megakaryocytogenesis.

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Increased D-type Cyclin Expression Together with Decreased cdc2 Activity Confers Megakaryocytic Differentiation of a Human Thrombopoietin-dependent Hematopoietic Cell Line

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