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BDNF, produced by a TPO-stimulated megakaryocytic cell line, regulates autocrine proliferation

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ABSTRACT

While human platelets release endogenous brain-derived neurotrophic factor (BDNF) upon activation, a previous report on MEG-01, a megakaryocytic cell line, found no trace of BDNF production, and the pathophysiological function of platelet BDNF has remained elusive. In the present study, we demonstrate that MEG-01 produces BDNF in the presence of TPO and that this serves to potentiate cell proliferation. Our *in vitro* findings suggest that BDNF regulates MEG-01 proliferation in an autocrine manner, and we suggest that BDNF may be a physiological autocrine regulator of megakaryocyte progenitors.

1. Introduction

Megakaryopoiesis involves proliferation, differentiation and maturation processes, which culminates in platelet production. Along the course, hematopoietic stem cells (HSC) shift from megakaryocyte (MK) progenitors, proMK (also known as MK precursors), to MK and platelets [1]. MK progenitors (BFU-MEG, CFU-MEG, megakaryoblast) undergo proliferation, termed clonal expansion. MK progenitors subsequently differentiate into proMK, which then enter the MK maturation phase. During the maturation phase, proMK undergo a polyploidization by multiple rounds of endoreplication. Fully mature MKs with high ploidy (more than 16*N*) and enlarged cytoplasm are capable of producing proplatelets and platelets [2].

In the MK development process, a number of cytokines are involved (e.g., thrombopoietin (TPO), stem cell factor (SCF), IL-3, IL-6, IL-11 and leukocyte inhibitory factor (LIF)). These factors are classified into two categories: megakaryocyte-colony stimulating factor (MEG-CSF) and megakaryocyte-potentiator (MEG-POT). IL-3 and SCF are classified as MEG-CSF and induce clonal expansion of MK progenitors, suppressing cell maturation such as polyploidization [3–5]. On the other hand, IL-6, IL-11 and LIF are categorized as MEG-POT, and give rise to both cell differentiation and polyploidization of MK progenitors [3]. TPO is the most potent stimulus to commit HSC to the MK lineage, and it is a bifunctional factor in terms of promoting clonal expansion and also maturation of MK lineages in conjunction with several cytokines [6,7]. However, attempts to produce large numbers of platelets from ES-, or iPS-derived MK with TPO and other cytokines have met with limited success, and it therefore seems most likely that other important factors are at work physiologically [8–12].

Brain-derived neurotrophic factor (BDNF), a member of the nerve growth factor family, is a basic, homodimeric cytokine (28 kDa; pI, 10.3) [13,14], and promotes fetal development of the central nervous system, neuron plasticity and preservation of memory [15,16]. The expression or secretion level of BDNF in the central nervous system is altered in neurodegenerative and psychiatric diseases [17–19]. In peripheral organs, BDNF is expressed in various tissues (e.g., thymus, liver, spleen, heart and lung), albeit to a limited degree [20–23]. In peripheral blood, it is known that platelets store large amounts of BDNF and release it upon stimulation by several agonists [24–26]. However, it has been considered

Abbreviations: BDNF, brain-derived neurotrophic factor; BFU-MK, burst forming unit-megakaryocyte; CFU-MK, colony forming unit-megakaryocyte; HSC, hematopoietic stem cell; TPO, thrombopoietin; MEG-CSF, megakaryocyte-colony stimulating factor; MEG-POT, megakaryocyte-potentiator; MK, megakaryocyte.

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that MKs lack the ability to produce BDNF, based on a previous report that MEG-01, a megakaryocytic cell line, often used as a MK *in vitro* model, neither expressed nor produced BDNF [25]. Thus, to date, the origin of platelet BDNF has remained elusive, and the pathophysiological roles of BDNF with regard to MK or platelet function have not been investigated.

In a previous study, we found that BDNF is released from platelets along with PAR-1 in preference to PAR-4 agonists. We also found that BDNF is present in the cytoplasm of platelets, as well as in α granules, suggesting that BDNF is either produced in platelets or passed down from megakaryocytes [26]. These findings are at odds with a previous report [25] that BDNF is not produced by megakaryocytes. We therefore sought to thoroughly investigate whether MKs can produce BDNF.

2. Material and methods

2.1. Cell culture

MEG-01 (subdivided from Health Science Research Resources Bank, Osaka, Japan) were cultured in RPMI 1640 (Sigma Aldrich, Tokyo, Japan) containing 10% FBS (Invitrogen, Life Technologies Corp., CA, USA) and penicillin/streptomycin solution (Sigma Aldrich) at 37 °C under a 5% CO_2 atmosphere.

2.2. Cell proliferation assay

Cells were stimulated with 50 ng/ml human TPO (R&D systems, MN, USA), 50 ng/ml human BDNF (Invitrogen) or 2.5 μg/ml BDNF neutralizing polyclonal antibody (G164B; Promega Corporation, WI, USA). At days 2, 4 and 6 after stimulation with several cytokines or antibody, the cells were counted using Improved Neubauer's Counting Chamber.

2.3. Western blotting analysis

Cells were lyzed with lysis buffer (20 mM Na_2HPO_4, 2 mM $NaH_2PO_4{\cdot}2H_2O$, 150 mM NaCl, 1.0% Triton X-100; pH 7.5) containing protease inhibitor mix (GE Healthcare Japan Corporation, Tokyo, Japan). Lysates were subjected to SDS–PAGE and electrotransferred to PVDF membranes (Hybond-P, GE healthcare). Primary antibodies were anti-BDNF rabbit polyclonal antibody (sc-546; Santa Cruz) and anti-β actin monoclonal antibody (Sigma Aldrich). Secondary antibodies were anti-rabbit goat HRP conjugate and anti-mouse goat HRP conjugate (GE healthcare Japan Corporation). Signals were detected by exposure to Hyperfilm ECL with ECL prime (GE healthcare Japan Corporation).

2.4. Polyploidization assay

Cells stimulated with TPO and/or BDNF or treated with corresponding antibodies were fixed with ice-cold 70% ethanol for more than 4 h, and DNA was subsequently stained by PI/RNase staining buffer (BD Biosciences, NJ, USA) according to the manufacturer's instruction. After being washed with PBS, the DNA contents were measured by Cytomics FC500 (Beckman Courter, Fullerton, CA, USA).

2.5. Statistical analysis

Values are shown as means ± S.E.M. Statistical analysis was performed using the Mann–Whitney *U*-test. Levels of significance were set at $p < 0.05$.

3. Results

3.1. BDNF produced by MEG-01 stimulated by TPO promotes MEG-01 proliferation, suggesting that BDNF serves as an autocrine factor for proliferation

While checking the experimental methods of the previous report, which negated BDNF production by MEG-01, we found that BDNF production had been assessed only under autonomous proliferating conditions, in the absence of cytokines [25]. On the other hand, *in vivo*, megakaryocytes in bone marrows should be stimulated by TPO and other cytokines [27]. We thus examined BDNF levels in MEG-01 with or without TPO stimulation. There was no BDNF signal in MEG-01 without TPO, whereas BDNF production was clearly detected in MEG-01 stimulated with TPO (Fig. 1A, upper panel). The intensity of BDNF in MEG-01 stimulated with TPO increased in a time-dependent manner for 6 days (Fig. 1A, lower panel). These findings suggest that TPO stimulation induces BDNF production in MEG-01.

We next investigated the physiological role of BDNF production in MEG-01. We assayed the effects alone or in combination with TPO and BDNF, on MEG-01 proliferation (Fig. 1B). TPO added to the cell culture increased the cell number, as expected. BDNF alone accelerated MEG-01 proliferation, and there was an additional effect when given in combination with TPO. The addition of TPO and BDNF increased the cell number by 177% over the number without TPO and BDNF, suggesting that they have potent stimulating effects on MEG-01 proliferation. To further examine the effect of BDNF on MEG-01 proliferation, we added a BDNF neutralizing antibody to MEG-01 cultured with or without TPO (Fig. 1C). As expected, cell proliferation was unaffected by the BDNF neutralizing antibody in MEG-01 cultured without TPO, since there was no BDNF production. However, in MEG-01 cultured with TPO, a BDNF neutralizing antibody significantly decreased the cell number ($p < 0.05$), suggesting that BDNF produced by TPO-stimulated MEG-01 enhanced its own proliferation, i.e., in an autocrine manner.

3.2. BDNF does not promote polyploidization of MEG-01

We next sought to determine whether BDNF has detectable MEG-POT activity, which can induce MK maturation such as polyploidization. In our polyploidization assay, TPO was considered as a positive control stimulus for inducing polyploidization in MEG-01. Polyploidy of cells cultured with several cytokines for 6 days was analyzed by FCM with PI staining. Mean ploidy at day 6 of MEG-01 with or without TPO and/or BDNF, TPO(−)/BDNF(−), TPO(+)/BDNF(−), TPO(−)/BDNF(+) and TPO(+)/BDNF(+), were 3.10 ± 0.24, 3.47 ± 0.42, 3.27 ± 0.44 and 3.28 ± 0.29, respectively (Fig. 2, upper panel). The mean ploidy of MEG-01 cultured with the cytokine combination including BDNF suggests that BDNF has a tendency to repress polyploidy, however this effect was not statistically significant (Fig. 2, lower panel). We also examined the mean ploidy of MEG-01 cultured with TPO and a BDNF neutralizing antibody, TPO(+)/n-Ab(+). Compared with MEG-01 with only TPO, TPO(+)/n-Ab(−), the marked difference of mean ploidy was not observed between these groups of MEG-01 (data not shown). These observations suggest that BDNF does not positively influence MEG-01 maturation events such as polyploidy.

4. Discussion

In the present study, we demonstrate that (1) MEG-01 produces BDNF upon TPO stimulation (2) BDNF has a proliferative effect on MEG-01 in an autocrine manner, and (3) BDNF does not positively affect polyploidization of MEG-01. To the best of our knowledge,

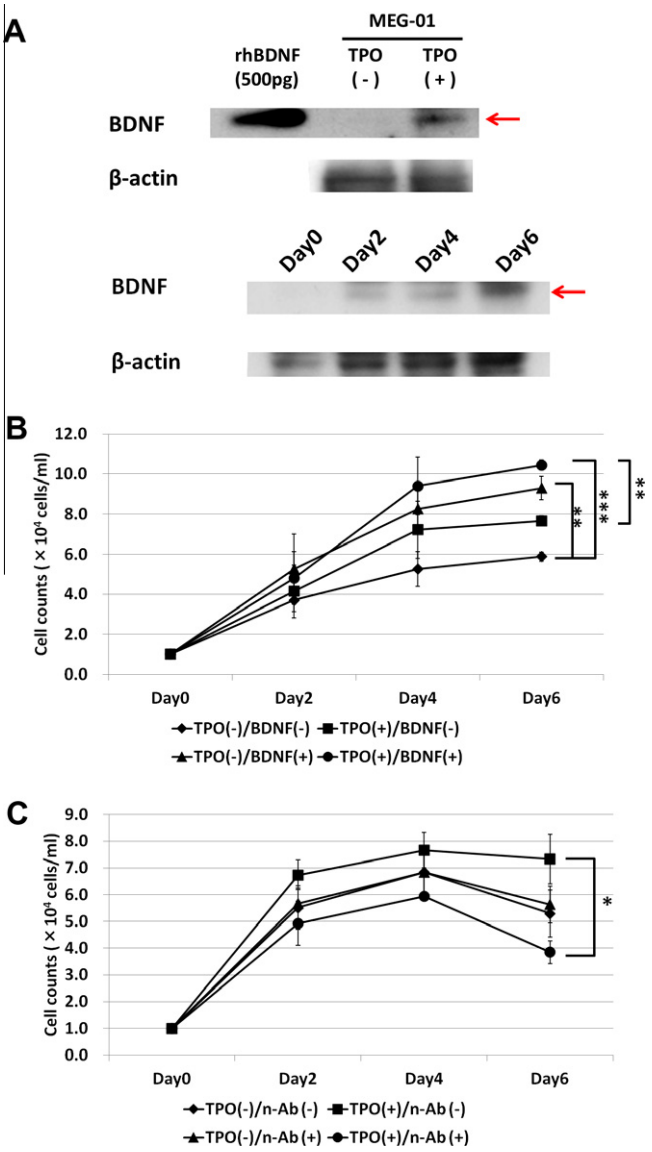

Fig. 1. TPO stimulation induces BDNF production in MEG-01, resulting in acceleration of cell proliferation. A. MEG-01 with TPO stimulation produces BDNF. MEG-01 was cultured with 50 ng/μl of TPO for 6 days and harvested on days 2, 4 and 6. Subsequently, cells were subjected to western blotting analysis. The arrow indicates signals for BDNF. Five hundred picograms of recombinant human BDNF (rhBDNF) was loaded as a positive control, and β-actin was used as a loading control. B. BDNF promotes MEG-01 proliferation. Cell proliferation assay was performed on MEG-01 in culture conditions alone or in combination with 50 ng/μl of TPO and 50 ng/μl of BDNF. Four groups of MEG-01, TPO(−)/BDNF(−), TPO(+)/BDNF(−), TPO(−)/BDNF(+) and TPO(+)/BDNF(+) were cultured for 6 days and cells were counted on days 2, 4 and 6. On day 6 the numbers of cells in MEG-01 with BDNF, TPO(−)/BDNF(+) and TPO(+)/BDNF(+) groups were significantly increased ($p < 0.01$ and $p < 0.001$, respectively). The combination of TPO and BDNF showed a significant increase in MEG-01 cell numbers as compared with TPO alone ($p < 0.01$). C. BDNF neutralizing antibody inhibits the proliferation of MEG-01 stimulated with TPO. MEG-01 with or without 50 ng/μl of TPO were cultured with 2.5 μg/ml of BDNF neutralizing antibody (n-Ab) for 6 days. On day 6, a significant difference was observed between TPO-stimulated MEG-01 with and without neutralizing antibody, TPO(+)/n-Ab(+) and TPO(+)/n-Ab(−), ($p < 0.05$). Each graph shows the average values from three independent experiments. Error bar indicates S.E.M. One, two, and three asterisks indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

these findings are the first report of the production of BDNF in a MK lineage cell line, and lead us to the hypothesis that BDNF potentiates the cell proliferation of MK lineages *in vivo*.

TPO is an important factor for maintaining megakaryopoiesis and induces up-regulation of megakaryopoietic cytokines in MK lineages [28–30]. In our experiments, we found that BDNF was produced by MEG-01 in the presence of TPO and the levels of BDNF in MEG-01 increased in culture time-dependently (Fig. 1A). Since HSCs and MK progenitors in the bone marrow are exposed to constitutive TPO supplied from the liver and kidney [27], our observations suggest that MK lineages might consistently produce BDNF *in vivo*.

In order to examine the pathophysiological functions of BDNF to MK lineages, we next investigated whether BDNF affected the cell proliferation and polyploidization of MEG-01. Our proliferation assay showed that BDNF obviously accelerated MEG-01 proliferation (Fig. 1B). Interestingly, BDNF alone gives rise to significant proliferation, and the combination dosage of TPO and BDNF shows a synergistic activity. Moreover, we found that the blocking of endogenous BDNF by a neutralizing antibody significantly reduced proliferation of TPO-stimulated MEG-01 (Fig. 1C), suggesting that BDNF acts in an autocrine manner to accelerate MEG-01 proliferation in the presence of TPO. It is of interest that the final cell number of MEG-01 cultured with TPO and a neutralizing BDNF antibody was less than that of MEG-01 cultured without TPO. While it is conceivable that TPO rendered MEG-01 partially BDNF-dependent for proliferation, there is no direct evidence to support or disprove this hypothesis, and therefore it remains to be elucidated. However, investigation of the TPO cascade, giving rise to BDNF production offers a clue in connection with our hypothesis. In addition, given the observation of BDNF autocrine stimulation of MEG-01, it is likely that there is a receptor for BDNF on the MEG-01 cell surface. However, in the present study, TrkB, a BDNF receptor that regulates cell proliferation and survival on neuronal cells [31], was not detected by WB analysis in MEG-01 with or without TPO stimulation (data not shown). Consistent with our data, previous reports have described that TrkB is not detected in human platelet [25,32]. From these previous reports and our observations, we consider that there is an unidentified novel receptor on MK or the platelet surface involve in the clonal expansion of MK progenitors.

In contrast with the proliferation activity, BDNF did not positively affect polyploidization of MEG-01 (Fig. 2). This observation characterizes the BDNF activity in megakaryopoiesis as being restricted to clonal expansion of MK progenitors, not in maturation. It has been reported that several cytokines (e.g., VEGF, angiopoietin and PDGF), which are induced by TPO in MK lineages, affect polyploidization and granulation, as MK maturation regulators [33–35]. On the other hand, factors that accelerate cell proliferation while repressing polyploidization of MK lineages have rarely been reported. Thus, we propose that BDNF is a unique and essential factor for MK progenitors to undergo clonal expansion.

The limitations of this study include only one experimental model of the megakaryocytic cell and application of a malignant MK lineage to examine BDNF activity in MK progenitors. Accordingly, experiments involving apply normal, non-malignant, MK lineages or HSCs from bone marrow are needed to assess the physiological functions of BDNF on megakaryopoiesis. In addition, to understand how BDNF regulates clonal expansion of MK progenitors, it is necessary to explore the mechanism of TPO-induced BDNF production, to identify the BDNF receptor on the MK surface, and to characterize the BDNF signaling pathway promoting MK progenitor proliferation.

Herein, we propose that BDNF is a potential factor regulating megakaryopoiesis. From our findings based on a megakaryocytic cell line model, we propose that BDNF is produced in MK lineages in the presence of TPO. The pathophysiological function of BDNF seems to be to act in an autocrine manner for the clonal expansion of MK progenitors while repressing maturation, like a MEG-CSF. Although there are some experimental limitations as mentioned above, the accumulation of knowledge between megakaryopoiesis

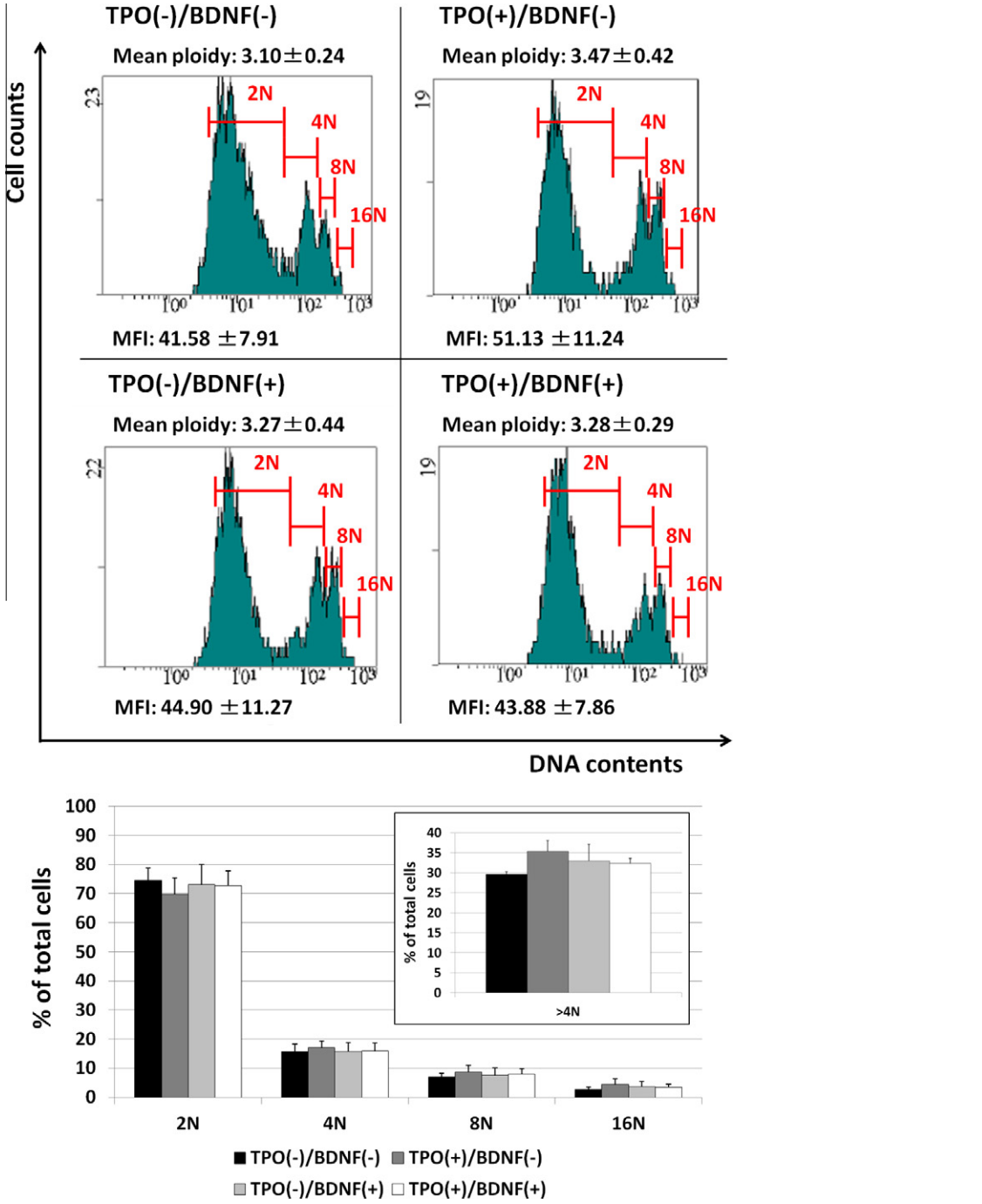

Fig. 2. BDNF does not positively affect polyploidization of MEG-01. MEG-01 was cultured with or without 50 ng/μl of TPO and/or 50 ng/μl of BDNF for 6 days. To evaluate mean ploidy, DNA content at day 6 of culture was measured by FCM analysis with PI staining. The representative histograms of DNA content for each cytokine combination group of MEG-01, TPO(−)/BDNF(−), TPO(+)/BDNF(−) and TPO(−)/BDNF(+) and TPO(+)/BDNF(+) are shown in the upper panel. Mean ploidy was calculated from percentages within four gates over the range of 2*N* to 16*N* as indicated in each histogram. Mean fluorescence intensities (MFI) of DNA content are also shown in each histogram. In addition, percentages of each nuclear lobe are shown in the lower panel. The inserted graph indicates the percentage of polyploided MEG-01 (>4*N*). For several end-point parameters; mean ploidy, MFI and percentages of polyploided cell, BDNF does not show a positive effect for MEG-01 polyploidization. Each values show the average plus or minus S.E.M from four independent experiments.

and BDNF would greatly contribute to the elucidation of the pathogenesis of thrombocytopenia or thrombocythemia. We consider that BDNF has great potential to be a novel therapeutic target for thrombocyte-associated disorders.

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References

[1] Y. Chang, D. Bluteau, N. Debili, W. Vainchenker, From hematopoietic stem cells to platelets, J. Thromb. Haemostasis 5 (Suppl. 1) (2007) 318–327.

[2] S.R. Patel, J.H. Hartwig, J.E. Italiano Jr., The biogenesis of platelets from megakaryocyte proplatelets, J. Clin. Invest. 115 (2005) 3348–3354.

[3] M. Tsujimoto, N. Tsuruoka, N. Ishida, T. Kurihara, F. Iwasa, K. Yamashiro, T. Rogi, S. Kodama, N. Katsuragi, M. Adachi, T. Katayama, M. Nakao, K. Yamaichi, J. Hashino, M. Haruyama, K. Miura, T. Nakanishi, H. Nakazato, M. Teramura, H. Mizoguchi, N. Yamaguchi, Purification, cDNA cloning, and characterization of a new serpin with megakaryocyte maturation activity, J. Biol. Chem. 272 (1997) 15373–15380.
[4] E. Sitnicka, N. Lin, G.V. Priestley, N. Fox, V.C. Broudy, N.S. Wolf, K. Kaushansky, The effect of thrombopoietin on the proliferation and differentiation of murine hematopoietic stem cells, Blood 87 (1996) 4998–5005.
[5] M. Kobayashi, J.H. Laver, T. Kato, H. Miyazaki, M. Ogawa, Thrombopoietin supports proliferation of human primitive hematopoietic cells in synergy with steel factor and/or interleukin-3, Blood 88 (1996) 429–436.
[6] K. Kaushansky, The molecular mechanisms that control thrombopoiesis, J. Clin. Invest. 115 (2005) 3339–3347.
[7] K. Kaushansky, J.G. Drachman, The molecular and cellular biology of thrombopoietin: the primary regulator of platelet production, Oncogene 21 (2002) 3359–3367.
[8] N. Takayama, S. Nishimura, S. Nakamura, T. Shimizu, R. Ohnishi, H. Endo, T. Yamaguchi, M. Otsu, K. Nishimura, M. Nakanishi, A. Sawaguchi, R. Nagai, K. Takahashi, S. Yamanaka, H. Nakauchi, K. Eto, Transient activation of c-MYC expression is critical for efficient platelet generation from human induced pluripotent stem cells, J. Exp. Med. 207 (2010) 2817–2830.
[9] K. Eto, R. Murphy, S.W. Kerrigan, A. Bertoni, H. Stuhlmann, T. Nakano, A.D. Leavitt, S.J. Shattil, Megakaryocytes derived from embryonic stem cells implicate CalDAG-GEFI in integrin signaling, Proc. Natl. Acad. Sci. USA 99 (2002) 12819–12824.
[10] N. Takayama, H. Nishikii, J. Usui, H. Tsukui, A. Sawaguchi, T. Hiroyama, K. Eto, H. Nakauchi, Generation of functional platelets from human embryonic stem cells in vitro via ES-sacs, VEGF-promoted structures that concentrate hematopoietic progenitors, Blood 111 (2008) 5298–5306.
[11] Y. Matsubara, E. Saito, H. Suzuki, N. Watanabe, M. Murata, Y. Ikeda, Generation of megakaryocytes and platelets from human subcutaneous adipose tissues, Biochem. Biophys. Res. Commun. 378 (2009) 716–720.
[12] M. Gaur, T. Kamata, S. Wang, B. Moran, S.J. Shattil, A.D. Leavitt, Megakaryocytes derived from human embryonic stem cells: a genetically tractable system to study megakaryocytopoiesis and integrin function, J. Thromb. Haemostasis 4 (2006) 436–442.
[13] Y.A. Barde, D. Edgar, H. Thoenen, Purification of a new neurotrophic factor from mammalian brain, EMBO J. 1 (1982) 549–553.
[14] J. Leibrock, F. Lottspeich, A. Hohn, M. Hofer, B. Hengerer, P. Masiakowski, H. Thoenen, Y.A. Barde, Molecular cloning and expression of brain-derived neurotrophic factor, Nature 341 (1989) 149–152.
[15] M. Bibel, Y.A. Barde, Neurotrophins: key regulators of cell fate and cell shape in the vertebrate nervous system, Genes Dev. 14 (2000) 2919–2937.
[16] D. Dawbarn, S.J. Allen, Neurotrophins and neurodegeneration, Neuropathol. Appl. Neurobiol. 29 (2003) 211–230.
[17] N. Durany, T. Michel, J. Kurt, F.F. Cruz-Sanchez, J. Cervos-Navarro, P. Riederer, Brain-derived neurotrophic factor and neurotrophin-3 levels in Alzheimer's disease brains, Int. J. Dev. Neurosci. 18 (2000) 807–813.
[18] M.E. Kozisek, D. Middlemas, D.B. Bylund, Brain-derived neurotrophic factor and its receptor tropomyosin-related kinase B in the mechanism of action of antidepressant therapies, Pharmacol. Ther. 117 (2008) 30–51.
[19] L. Tapia-Arancibia, E. Aliaga, M. Silhol, S. Arancibia, New insights into brain BDNF function in normal aging and Alzheimer disease, Brain Res. Rev. 59 (2008) 201–220.
[20] P. Ernfors, C. Wetmore, L. Olson, H. Persson, Identification of cells in rat brain and peripheral tissues expressing mRNA for members of the nerve growth factor family, Neuron 5 (1990) 511–526.
[21] P.C. Maisonpierre, L. Belluscio, B. Friedman, R.F. Alderson, S.J. Wiegand, M.E. Furth, R.M. Lindsay, G.D. Yancopoulos, NT-3, BDNF, and NGF in the developing rat nervous system: parallel as well as reciprocal patterns of expression, Neuron 5 (1990) 501–509.
[22] P.C. Maisonpierre, M.M. Le Beau, R. Espinosa 3rd, N.Y. Ip, L. Belluscio, S.M. de la Monte, S. Squinto, M.E. Furth, G.D. Yancopoulos, Human and rat brain-derived neurotrophic factor and neurotrophin-3: gene structures, distributions, and chromosomal localizations, Genomics 10 (1991) 558–568.
[23] R. Katoh-Semba, I.K. Takeuchi, R. Semba, K. Kato, Distribution of brain-derived neurotrophic factor in rats and its changes with development in the brain, J. Neurochem. 69 (1997) 34–42.
[24] H. Yamamoto, M.E. Gurney, Human platelets contain brain-derived neurotrophic factor, J. Neurosci. 10 (1990) 3469–3478.
[25] H. Fujimura, C.A. Altar, R. Chen, T. Nakamura, T. Nakahashi, J. Kambayashi, B. Sun, N.N. Tandon, Brain-derived neurotrophic factor is stored in human platelets and released by agonist stimulation, Thromb. Haemostasis 87 (2002) 728–734.
[26] S. Tamura, H. Suzuki, Y. Hirowatari, M. Hatase, A. Nagasawa, K. Matsuno, S. Kobayashi, T. Moriyama, Release reaction of brain-derived neurotrophic factor (BDNF) through PAR1 activation and its two distinct pools in human platelets, Thromb. Res. 128 (2011) e55–e61.
[27] K. Kaushansky, Lineage-specific hematopoietic growth factors, N. Engl. J. Med. 354 (2006) 2034–2045.
[28] S. Jeanpierre, F.E. Nicolini, B. Kaniewski, C. Dumontet, R. Rimokh, A. Puisieux, V. Maguer-Satta, BMP4 regulation of human megakaryocytic differentiation is involved in thrombopoietin signaling, Blood 112 (2008) 3154–3163.
[29] D. Bonci, M. Hahne, N. Felli, C. Peschle, R. De Maria, Potential role of APRIL as autocrine growth factor for megakaryocytopoiesis, Blood 104 (2004) 3169–3172.
[30] E. Saulle, R. Guerriero, A. Petronelli, E. Coppotelli, M. Gabbianelli, O. Morsilli, I. Spinello, E. Pelosi, G. Castelli, U. Testa, S. Coppola, Autocrine role of angiopoietins during megakaryocytic differentiation, PLoS One 7 (2012) e39796.
[31] J. Soule, E. Messaoudi, C.R. Bramham, Brain-derived neurotrophic factor and control of synaptic consolidation in the adult brain, Biochem. Soc. Trans. 34 (2006) 600–604.
[32] T. Burnouf, Y.P. Kuo, D. Blum, S. Burnouf, C.Y. Su, Human platelet concentrates: a source of solvent/detergent-treated highly enriched brain-derived neurotrophic factor, Transfusion 52 (2012) 1721–1728.
[33] C.P. Tseng, P. Chang, C.L. Huang, J.C. Cheng, S.S. Chang, Autocrine signaling of platelet-derived growth factor regulates disabled-2 expression during megakaryocytic differentiation of K562 cells, FEBS Lett. 579 (2005) 4395–4401.
[34] I. Casella, T. Feccia, C. Chelucci, P. Samoggia, G. Castelli, R. Guerriero, I. Parolini, E. Petrucci, E. Pelosi, O. Morsilli, M. Gabbianelli, U. Testa, C. Peschle, Autocrine-paracrine VEGF loops potentiate the maturation of megakaryocytic precursors through Flt1 receptor, Blood 101 (2003) 1316–1323.
[35] S. Coppola, L. Narciso, T. Feccia, D. Bonci, L. Calabro, O. Morsilli, M. Gabbianelli, R. De Maria, U. Testa, C. Peschle, Enforced expression of KDR receptor promotes proliferation, survival and megakaryocytic differentiation of TF1 progenitor cell line, Cell Death Differ. 13 (2006) 61–74.