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Letter to the Editors-in-Chief

Pituitary adenylate cyclase-activating polypeptide (PACAP): Differential effects on neonatal vs adult megakaryocytopoiesis

Pituitary adenylate cyclase-activating polypeptide (PACAP), a neuroendocrine hormone of the secretin peptide superfamily, is mainly produced in hypothalamus. PACAP shows diverse effects on CNS, peripheral organs, and some hematopoietic cells by VPAC1 and 2 receptor signaling [1]. Megakaryocytes (MKs) express the stimulatory G protein (Gs)-coupled PACAP/VIP-indifferent VPAC1 receptor. Previously, Peeters et al. [2] reported the inhibitory effects of PACAP receptor on in vitro megakaryocyte differentiation. Recently, it was observed that PACAP contribute to the proliferation of hematopoietic progenitor cells in murine bone marrow by PACAP specific receptor [3]. In our study on human MK development, we found that PACAP receptor-VPAC1 is differentially regulated in MKs through development. Considering differential expression of PACAP receptor-VPAC1 in human MK development, we hypothesized that PACAP may have different effects on MK differentiation from neonatal (CB)- and adults (PB)-derived CD34+ hematopoietic progenitors.

Thrombocytopenia, the deficiency of platelets (platelet count $< 150 \times 10^9$/l) in the blood, is a major clinical problem encountered in infants admitted to Neonatal Intensive Care Unit (NICU); this hemostatic abnormality is more common in all sick, preterm and low birth weight neonates, and play a major role as co-factor in the incidence and severity of neonatal intra-ventricular hemorrhages (IVH) [4], which is a leading cause of poor neurological outcome and mortality in sick neonates [4–6]. The cause for this predisposition is unclear, however we and many other research groups have observed that neonatal MKs are developmentally different from adult's MKs. Specifically, neonatal MK progenitors (MP) are hyper-proliferative and produce smaller and low ploidy MKs as compared to adults. These developmental differences may contribute to this predisposition in neonatal MKs [7–9].

To test the developmental differences in the expression levels of PACAP receptor-VPAC1 in neonatal vs adult's MKs, we cultured human CB- and PB-CD34+ cells ($n = 3$ for each group) in serum-free medium with thrombopoietin (50 ng/mL), as previously described [10]. After 14 day of culture Flow cytometry analysis was performed using human anti-CD42/APC to monitor thrombopoietin stimulated MKs differentiation. We observed that CD34+ cells from CB generated approximately 2-fold more CD42 positive MKs as compared to PB-CD34+ cells ($n = 3$, $P < .02$; Fig. 1A), however the change in CD42 mean fluorescence density was higher in PB-MKs as compared to CB-MKs on day 14 ($n = 3$, $P < .05$; Fig. 1A). Further, we observed the PACAP receptor-VPAC1 mRNA expression in neonatal- and adult-CD34+ progenitor-derived MKs and resulted in approximately 6 fold higher expression levels in CB- as compared to PB-MKs ($n = 3$, $P < .02$; Fig. 1B). However, because of sample limitations, the protein content of PACAP receptor VPAC1 was not measured in current study. Finally, to study the effect of PACAP on in vitro MK differentiation from CB- and PB-CD34+ hematopoietic progenitors, we cultured CB- and PB-CD34+ hematopoietic progenitors for 14 days with or without PACAP (1 μM) (TOCRIS, Cat # 1186) in the presence of TPO (50 ng/mL) in serum-free medium ($n = 3$ for each group). After 14 days, Flow Cytometry analysis was performed using human anti-CD41/FITC and anti-CD42/APC to monitor the number of differentiated MKs. The CD41 and CD42 positive CB- and PB-MK numbers in presence of PACAP were expressed as relative values to their controls (without PACAP) at the same stage of culture. The number of CB-CD41 positive MKs was significantly reduced in the presence of PACAP compared to their control ($n = 3$, $P < .05$; Fig. 1C), whereas PB-CD41 positive MKs were not affected significantly ($n = 3$, $P > .05$; Fig. 1C). Similarly, we also observed about 2 fold decrease in the number of CB-CD42+ MKs in presence of PAPAC as compared to control (n = 3, $P < .05$; Fig. 1D), whereas the decrease in the number of PB-CD42+ MKs was not significant (n = 3, $P > .05$; Fig. 1D).

Over the past two decades, observations from different research groups have proven that human neonatal- and adult-MKs are developmentally different [7–10]. In the same line, we have also observed developmental differences in neonatal vs adult MKs. Current study results are also suggesting that human CB and PB MKs have substantially different biological responses to PACAP. In current study, we observed that PACAP is a negative regulator of MK differentiation, as previously reported [2]. However, the negative effects of PACAP were more drastic on MK differentiation process from CB-CD34+ hematopoietic progenitors as compared to MK differentiation from PB-CD34+ hematopoietic progenitors (Fig. 1C and D). Further, we have also observed developmental differences in the expression levels of PACAP receptor-VPAC1 in neonatal vs adults MKs and found about 6 fold higher expressions in neonatal MKs as compared to adults. Hypothetically, the higher expression levels of PACAP receptor-VPAC1 in neonatal MKs might contribute more to the inhibitory effects of PACAP on neonatal MK differentiation, as we have observed in current study.

In conclusion, our results demonstrate that PACAP is an inhibitor of MK differentiation however the negative effects are more drastic on neonatal MK differentiation as compared to adults. Higher expression of the PACAP receptor-VPAC1 in neonatal MKs possibly contributes to the predisposition of neonatal MKs via PACAP/VPAC1 signaling mechanism.

Conflicts of interest

The authors declare that they have no conflict of interest.

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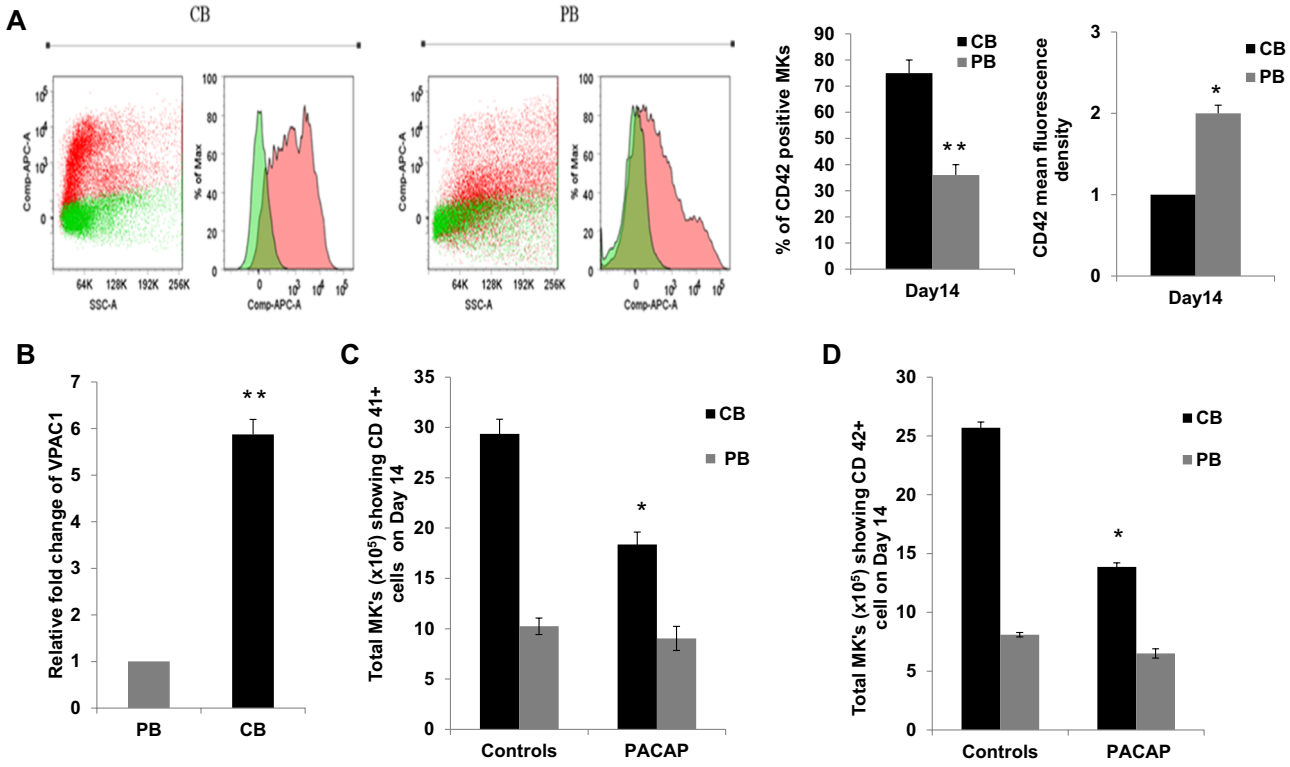

Fig. 1. Effects of PACAP on in vitro megakaryocytopoiesis. (A) Flow cytometry analysis of the expression of megakaryocytic marker-CD42 assessed after 14 days of culture of the CB- and PB-CD34+ hematopoietic progenitors in medium containing 50 ng of thrombopoietin per ml ($n = 3$, $^{**}p = .02$; $^{*}p = .05$). (B) VPAC1 mRNA levels were determined by QRT-PCR in CB and PB derived MKs. VPAC1 levels in MKs derived from CB expressed as relative to VPAC1 levels in PB derived MKs at day 14 (n = 3, $^{**}p = .02$). (C and D) The inhibitory effect of PACAP was observed on thrombopoietin-induced megakaryocytopoiesis by Flow cytometry. The number of (C) CD41+ and (D) CD42+ MKs, derived from CB- and PB-CD34+ progenitor, in presence of PACAP, was expressed as relative to their control (without PACAP) (n = 3, $^{*}p < .05$). Bars represent mean ± SD of three independent experiments.

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