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Prostaglandin E2 regulates vertebrate haematopoietic stem cell homeostasis

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Abstract

Haematopoietic stem cell (HSC) homeostasis is tightly controlled by growth factors, signalling molecules and transcription factors. Definitive HSCs derived during embryogenesis in the aorta-gonad-mesonephros region subsequently colonize fetal and adult haematopoietic organs[1,2]. To identify new modulators of HSC formation and homeostasis, a panel of biologically active compounds was screened for effects on stem cell induction in the zebrafish aorta-gonad-mesonephros region. Here, we show that chemicals that enhance prostaglandin (PG) E2 synthesis increased HSC numbers, and those that block prostaglandin synthesis decreased stem cell numbers. The cyclooxygenases responsible for PGE2 synthesis were required for HSC formation. A stable derivative of PGE2 improved kidney marrow recovery following irradiation injury in the adult zebrafish. In murine embryonic stem cell differentiation assays, PGE2 caused amplification of multipotent progenitors. Furthermore, *ex vivo* exposure to stabilized PGE2 enhanced spleen colony forming units at day 12 post transplant and increased the frequency of long-term repopulating HSCs present in murine bone marrow after limiting dilution competitive transplantation. The conserved role for PGE2 in the regulation of vertebrate HSC homeostasis indicates that modulation of the prostaglandin pathway may facilitate expansion of HSC number for therapeutic purposes.

A chemical genetic screen was conducted to identify new pathways modulating definitive HSC formation during zebrafish embryogenesis. *runx1* and *cmyb*, required for mammalian HSC development, are expressed in the ventral wall of the dorsal aorta in a region analogous to the

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mammalian aorta-gonad-mesonephros (AGM) at 36 h post fertilization (h.p.f.)[3-5]. Wild-type embryos, incubated with individual chemicals, were examined for alterations in *runx1*$^{+}$/*cmyb*$^{+}$ HSCs by *in situ* hybridization expression at 36 h.p.f. A high percentage of compounds (91.7%, 2,275 of 2,357) failed to alter HSC expression, whereas 35 (1.4%) and 47 (1.9%) led to increased or decreased numbers of HSCs, respectively. Among these substances, 10 affected the prostaglandin pathway (Supplementary Table 1). *runx1*$^{+}$/*cmyb*$^{+}$ HSCs comprise a line of flattened endothelial cells (arrow) and haematopoietic clusters (arrowhead) in the aorta (Fig. 1a-c); linoleic acid increased HSC numbers (22 altered out of 30 scored), whereas celecoxib, a cyclooxygenase (Cox)2 inhibitor, decreased HSCs (26/31). PGE2 is the main effector prostanoid produced in the zebrafish[6] and is regulated by both Cox1 (also known as Ptgs1) and Cox2 (also known as Ptgs2a). Treatment of zebrafish embryos with PGE2 increased expression of *runx1/cmyb* (25/49), whereas Cox inhibition with SC560 (Cox1) and NS398 (Cox2) (Supplementary Fig. 1a-e) decreased HSC numbers in 30/36 and 35/44 cases, respectively. These findings argue persuasively for a specific role of PGE2 in the formation of AGM HSCs.

Cox1 is required for the development of the aorta-vein endothelial boundary during zebrafish development[7]; thus, alteration in Cox1 activity could have an impact on endothelial-derived HSCs. By *in situ* hybridization, *cox2* was diffusely expressed in the tail region at 36 h.p.f. (Supplementary Fig. 1f, g). In FACS-isolated blood and endothelial cell populations, both *cox1* and *cox2* were found to be highly expressed during the onset of definitive haematopoiesis. *cox1* was detected in both Lmo2^{+} endothelial cells and in Cd41^{+} HSCs, whereas *cox2* was only found in HSCs (Supplementary Fig. 1h). This suggests that Cox1 and Cox2 participate in HSC induction through regulation of the stem cell niche and the HSC itself.

A long-acting derivative of PGE2, 16,16-dimethyl-PGE2 (dmPGE2) caused an increase in *runx1*$^{+}$/*cmyb*$^{+}$ AGM HSCs in 78% of embryos (97/124) (Fig. 1e, h), whereas HSCs were inhibited by indomethacin (10 μM) treatment in 90% of embryos (92/102) (Fig. 1k and Supplementary Fig. 1j-r). Mass spectrometry of 36 h.p.f. embryos demonstrated that indomethacin treatment depressed PGE2 formation below detectable levels (from 18±6 pg per 50 embryos to <2 pg per 50 embryos; $n = 3$)[6]. dmPGE2 had minimal effects on the vasculature, as shown by *flk1* staining (Fig. 1f, i) whereas indomethacin slightly altered the intersomitic vessels in 30% (15/49) of embryos (Fig. 1l). At 36 h.p.f., live bigenic *cmyb-gfp; lmo2-dsRed* (green-fluorescent-protein-labelled HSCs and progenitors; red-labelled HSCs and endothelium) embryos imaged by confocal microscopy exhibited significantly decreased numbers of HSCs (yellow) following indomethacin treatment, and significantly increased HSCs after dmPGE2 exposure (Fig. 1g, j, m and Supplementary Fig. 1i). Quantitative PCR confirmed an enhancement in *runx1* and *cmyb* expression by dmPGE2, whereas indomethacin significantly reduced the expression of each gene (Fig. 1d).

To confirm the requirement of PGE2 activity, we used morpholino oligonucleotides to knock down expression of Cox1 and Cox2; a low dose (40 μM) inhibition of Cox1 minimizes toxicity, while mimicking Cox-dependent developmental defects[6]. Morpholino oligonucleotide knockdown of Cox1/Cox2 decreased the levels of prostaglandins and inhibited AGM HSCs (Cox1, 54/74; Cox2, 60/71) (Supplementary Fig. 1s-u). The morpholino-mediated effects on HSCs were reversed by dmPGE2 (Cox1 + dmPGE2, 29/52 rescued; Cox2 + dmPGE2, 43/60) (Supplementary Fig. 1y, z, a'). dmPGE2 rescued (25/45) morpholino-mediated knockdown of PGE2 synthase (35/50) indicating that PGE2 signalling was sufficient to modulate HSC formation (Supplementary Fig. 1u, b'). PGE2 signals through receptors Ptger1l-Ptger4l (ref. [8]). Morpholino-mediated knockdown of Ptger2l and Ptger4l diminished *runx1/cmyb* expression (Ptger2l, 39/63; Ptger4l, 44/67) and was not reversed by dmPGE2 (Supplementary Fig. 1s, t, c', d'). Quantitative PCR analysis showed *ptger2l/ptger4l* are present in HSCs (Supplementary Fig. 1e'). These experiments confirm that PGE2-mediated signalling regulates the formation of HSCs in the AGM region.

To examine the role of PGE2 in HSC homeostasis in adult zebrafish, we performed a kidney marrow irradiation-recovery assay in sublethally irradiated wild-type fish[9] (Fig. 2a). The rate of kidney marrow repopulation was significantly enhanced after exposure to 50 μM dmPGE2 (Fig. 2a, b), with progenitor recovery preceding reconstitution of the myeloid and lymphoid populations. Significant upregulation of stem, progenitor and endothelial cell markers was found after dmPGE2 treatment (Supplementary Fig. 2a). Inhibition of Cox activity significantly decreased kidney marrow recovery and affected overall survival (Supplementary Fig. 2b). Our results indicate that PGE2 has an important role in kidney marrow homeostasis.

We then evaluated the effects of PGE2 on murine HSC and progenitor populations. Addition of dmPGE2 to embryonic stem cells during embryoid body expansion increased haematopoietic colonies on an OP9 stromal cell layer and in methylcellulose assays[10] (Fig. 3a, b). OP9, definitive erythroid and granulocyte/monocyte colonies increased in a dose-dependent manner after exposure to 10 μM (granulocyte/monocyte, $P = 0.005$) and 20 μM (OP9, $P = 0.047$; definitive erythroid, $P = 0.04$; granulocyte/monocyte, $P = 0.007$) dmPGE2. The number of multipotent granulocyte/erythrocyte/monocyte/macrophage colonies was enhanced 2.9-fold following dmPGE2 treatment (10 μM, $P = 0.017$; 20 μM, $P = 0.016$). *Cox1* (also known as *Ptgs1*), *Cox2* (also known as *Ptgs2*), PGE2 synthase (*Ptges*) and *Ptger1-Ptger4* were present in embryonic stem cells at all stages examined (Supplementary Fig. 3a). Indomethacin inhibited colony growth at 20 μM (OP9, $P = 0.069$) and 100 μM (granulocyte/monocyte, $P = 0.024$) (Fig. 3a, b) and could be rescued by dmPGE2 (Supplementary Fig. 3b, c). These data suggest the role of PGE2 in regulating haematopoiesis is conserved between zebrafish and mammals.

To explore effects in an intact mammalian model, murine whole bone marrow (WBM) was exposed *ex vivo* to dmPGE2 (1 μM per 10^6 cells) and irradiated recipients were transplanted with 6×10^4 treated WBM cells. The number of spleen colony-forming units at day 12 post transplant (CFU-S12) was increased threefold ($P < 0.0001$) in recipients of dmPGE2-treated WBM (Fig. 4b, Supplementary Fig. 4b, Supplementary Table 6); numbers of more mature CFU-S8 colonies were also enhanced (Fig. 4a, Supplementary Fig. 3a, Supplementary Table 5). To assess the endogenous PGE2 requirement, WBM cells were incubated with indomethacin (1 μM 10^{-6} cells) or specific COX1 and COX2 inhibitors. After transplantation of 1×10^5 cells, a significant decrease ($P = 0.0001$) in the number of CFU-S12 was observed (Fig. 4c; Supplementary Fig. 4c, k, l; Supplementary Table 6). These results suggest that PGE2 enhances haematopoietic progenitor formation, and is required for CFU-S activity.

The prostaglandin pathway components are present in both stromal cell and HSC populations in mice and humans[11,12]. Cox1, Cox2, PGE2-synthase, Ptger2 and Ptger4 are present in fetal liver HSCs and in bone marrow HSCs after 5-fluorouracil (5-FU) injury, suggesting PGE2 signalling functions in murine HSCs[13]. To determine if the increase in CFU-S number is due to a direct effect of PGE2 on the stem/progenitor cell population, FACS-isolated $cKit^+Sca1^+$ $Lineage^-$ (KSL) bone marrow cells were exposed to dmPGE2 and transplanted into irradiated recipients. Both splenic weight (Supplementary Fig. 4d) and CFU-S12 were significantly increased, indicating that dmPGE2 can lead to cell-autonomous activation of HSCs and immature progenitors (Fig. 4d, Supplementary Table 6).

A limiting dilution competitive repopulation analysis was conducted to determine the effects of dmPGE2 on HSC reconstitution[14]. WBM (CD45.1) exposed to dmPGE2 *ex vivo* was mixed independently at varying doses with a fixed number of untreated competitor cells (CD45.1/CD45.2) and injected into congenic recipient mice (CD45.2). Peripheral blood obtained at 6, 12 and 24 weeks post transplantation was examined by FACS to determine percentage test-cell contribution to haematopoietic repopulation (Supplementary Fig. 4e-j); positive reconstitution was defined as test-cell multilineage chimaerism >5% (Supplementary Fig. 4f, h, i). A significant increase in the number of repopulating cells as determined by Poisson

statistics was seen in dmPGE2-treated bone marrow (Fig. 4e, Supplementary Fig. 4g, j). At 6 weeks, the calculated frequency of engrafting cells per 10^6 WBM cells was enhanced 3.3-fold ($P = 0.005$) in dmPGE2-treated WBM recipients, and the frequency of short-term repopulating HSCs was 4.0-fold ($P = 0.002$) higher at 12-weeks post-transplantation (Fig. 4e, f, Supplementary Fig. 4g). At 24 weeks, the frequency of long-term repopulating HSCs was 2.3-fold enhanced ($P = 0.05$) in recipients of dmPGE2-treated cells (Fig. 4f; Supplementary Fig. 4j). dmPGE2 treatment increased the frequency of repopulating HSCs in the mouse, but did not impair the differentiative capacity as seen by multilineage analysis. To determine whether dmPGE2 treatment enhanced homing to the bone marrow niche, WBM was labelled with a vital dye, CDFA, before transplantation; no significant difference in homing could be detected ($P = 0.83$) (Supplementary Fig. 5).

Here we have demonstrated that PGE2 enhances the number of HSCs and multipotent progenitors in two vertebrate species, zebrafish and mice. Prior studies have documented that unmodified PGE2 impairs blood-cell maturation in the mouse[15,16] and cell cycle stimulation in CFU-S8 progenitors[17]; however, the effects of prostaglandin-mediated cell signalling on HSCs have not been examined previously. Cox1 and Cox2 seem to have distinct functions in AGM HSC formation: Cox1 is important in the formation of the haematopoietic niche, whereas Cox2 is probably involved in self-renewal and proliferation of HSCs themselves. Conversely, homozygous *Cox1* or *Cox2* knockout mice are viable, without apparent defects in HSC formation[18], due to maternal and sibling PGE2 contribution[7,19]. Analyses of *Cox2*$^{-/-}$ mice demonstrated alterations in haematocrit levels and an impaired recovery from 5-FU-induced bone marrow injury[20]; these findings imply HSC defects in adult *Cox2*$^{-/-}$ mice that are compatible with our proposed role for prostaglandin in HSC homeostasis. To clarify the roles of COX1 and COX2 in regulating HSC homeostasis, we performed CFU-S12 (Fig. 4k, l) and 5-FU bone marrow recovery assays using selective inhibitors of COX1 (SC560) or COX2 (NS398). Inhibition of either enzyme significantly diminished CFU-S activity, as well as the recovery of peripheral blood and bone marrow WBC numbers (Supplementary Fig. 4m, n). Administration of dmPGE2 following 5-FU treatment significantly enhanced bone marrow recovery. These data suggest that both COX1 and COX2 have a role in regulating HSC homeostasis in adult mice, as in the zebrafish, and that PGE2 is the mediator of this HSC regulation. The precise mechanism of PGE2 modulation of vertebrate HSC homeostasis remains to be elucidated.

Patients undergoing bone marrow transplantation show increased endogenous PGE2 levels[21]. Our studies raise the possibility that administration of COX inhibitors following human bone marrow transplantation might impair HSC engraftment and result in delayed recovery of the WBC counts, in addition to adversely affecting platelet function. PGE2 and its analogues are safely administered to patients[22,23]. The concentration of dmPGE2 used to expand the number of murine HSCs falls within the physiological range of PGE2 in human serum[24], thus dmPGE2 or its derivatives may be useful for *ex vivo* or *in vivo* expansion of HSCs. Our studies illustrate that PGE2 functions as a potent regulator of HSCs in vertebrates, and may prove useful in treating patients with bone marrow failure or following transplantation.

METHODS SUMMARY

Wild-type age-matched embryos were exposed to individual test compounds from 3-somites until 36 h.p.f. and effects on HSCs were evaluated by *in situ* hybridization for *runx1* and *cmyb*. Treatment with PGE2, dmPGE2 and Cox inhibitors (indomethacin, SC560, NS398) at 10 μM was used to confirm and quantify the effects of prostaglandin signalling on HSCs by *in situ* hybridization, quantitative PCR[9] and confocal microscopy[25]. Expression of prostaglandin pathway components in HSCs was characterized by microarray analysis[26], quantitative PCR and *in situ* hybridization. Morpholino knockdown of prostaglandin pathway

components[6,8,27], and subsequent rescue by dmPGE2, was used to confirm the specificity of the results of the chemical treatments. Functional inhibition of prostaglandin synthesis was measured by mass spectroscopy for chemical and morpholino experiments. A flow-cytometry-based irradiation recovery assay was used to assess the impact of PGE2-mediated signalling on adult kidney marrow[28]. The effect of dmPGE2 and indomethacin on haematopoietic colony forming potential of embryonic stem cells was analysed by standard OP9 and methylcellulose colony forming assays[29,30]. CFU-S assays and limiting dilution competitive transplantation assays were used to test the effects of *ex vivo* dmPGE2 treatment or COX inhibition on haematopoietic stem and progenitor populations. Bone marrow ablation[13] by 5-FU was used to test the *in vivo* effect of dmPGE2 or COX inhibitor treatment on haematopoietic recovery in mammals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix

METHODS

Chemical screen design and confirmatory testing

Wild-type age-matched embryos were arrayed into 48-well plates (~5 embryos per well) of individual test compounds and exposed from 3-somites until 36 h.p.f. Three compound libraries were used: NINDS Custom Collection (1,040 compounds), SpecPlus Collection (960) and BIOMOL ICCB Known Bioactives (480). Five per cent (123/2480) of the compounds were toxic, resulting in death or severe morphological abnormalities. *In situ* hybridization for *runx1* and *cmyb* was performed to assess HSC numbers. Compounds were retested at 10, 20 and 50 μM. Stem cell specificity was assessed using *flk1* at 36 h.p.f. PGE2, PGI2, dmPGE2 and all Cox inhibitors (Sigma) were used at 10 to 20 μM.

Qualitative scoring (number of embryos with altered HSCs per number scored) of *runx1/cmyb* was conducted using the following criteria: Normal/unchanged, continuous line of *runx1*$^+$/*cmyb*$^+$ endothelial cells and occasional haematopoietic clusters; decreased/absent, reduction in *runx1*$^+$/*cmyb*$^+$ cells, including the presence of large gaps in the line of HSCs, isolated positive cells, or absence of expression; increased/excess, enhancement in *runx1*$^+$/*cmyb*$^+$ cells, including many HSC clusters, a thickened line of HSCs, or ectopic expression.

Confocal imaging

Live 36 h.p.f. treated bigenic zebrafish embryos were embedded in 1% low-melting point agarose containing 0.04 mg ml^{-1} Tricaine-S for confocal imaging. *lmo2-dsRed* fish were created as described[25]. *cmyb-gfp* transgenic reporter lines were created by homologous recombination of a 3.7 kb *EGFP* construct downstream of the 5′ untranslated region and precisely before the start site of a PAC clone containing *cmyb* (J. Galloway, H. Zhu, S. Lin and

L.I.Z., unpublished data). For HSC quantification, cMyb$^+$/Lmo2$^+$ cells were counted in projections of *z*-stack images (n = 10 per treatment).

Morpholino knockdown

Morpholino oligonucleotides (GeneTools) directed against zebrafish *cox1* and *cox2*, PGE2 synthase, and *ptger2l* and *ptger4l* (refs [6, 8, 27]) were injected (40 μM) into zebrafish embryos at the one-cell stage. For rescue experiments, 3-somite-stage morpholino-injected embryos were exposed to 10 μM dmPGE2.

Microarray gene expression profiling

Gata1-Gfp$^+$ (12 somites), Lmo2-Gfp$^+$ (12 somites and 35 h.p.f.) and Cd41-Gfp$^+$ (35 h.p.f.) cells were FACS-sorted; total RNA was purified and analysed using Affymetrix zebrafish gene chips as described previously[26].

Quantitative PCR

Quantitative PCR (qPCR) was performed using previously described primer sets[9]. Embryos (n = 50) were treated as described. qPCR (60 °C annealing) was performed using SYBR Green Supermix on the iQ5 Multicolour RTPCR Detection System (BioRad) (n = 10 replicates) and relative expression levels were determined. Primer pairs for *Ptger2* and *Ptger4* are shown (Supplementary Table 10). qPCR of whole kidney marrow RNA (n = 15 per variable) was performed on day 3 post irradiation as described. qPCR on S cell RNA (harvested in Stat-60, Tel-Test) was performed using the Stratagene Sybrgreen kit on the Stratagene qPCR machine. Prostaglandin pathway component primer sequences are shown (Supplementary Table 10).

Mass spectroscopy

PGE_2 and the stable PGI_2 metabolite, 6-keto-$PGF_{1\alpha}$, were measured using high-performance liquid chromatography-tandem mass spectrometry. Ethylacetate extracts from homogenized embryos were spiked with the corresponding stable-isotope-labelled internal standards (d_4-PGE_2 and d_4-6-keto $PGF_{1\alpha}$) and allowed to react with methoxylamine. The following mass transitions were monitored: *m/z* 384→272 (PGE), *m/z* 398→368 (6-keto $PGF_{1\alpha}$ and TxB2).

Irradiation recovery assay

Adult zebrafish were exposed to 23 Gy of γ-irradiation. On day 2 post irradiation, fish were exposed overnight to DMSO control, dmPGE2 (10 or 50 μM), indomethacin (10 μM), SC560 (10 μM) or NS398 (10 μM) in fish water. Whole kidney marrow isolated on days 0, 2, 4, 7, 10, 14 was subjected to forward scatter/side scatter (FSC/SSC) FACS analysis to identify haematopoietic lineages (n = 5 per treatment, 3 replicates)[28].

Embryonic stem cell differentiation assays

Embryonic stem cell haematopoietic differentiation assays were performed as previously described[29,30]. dmPGE2 (10, 20 or 100 μM) or indomethacin (20, 100 μM) were added at day 4 and 5 during embryoid body expansion. M3434 methylcellulose colony forming and OP9 colony assays were conducted on day 6 and analysed at days 8 and 5, respectively. Colony type was identified by morphological analysis; duplicate chemical exposures were averaged to determine the reported colony number (n = 3 replicates minimum).

Murine colony-forming units-spleen (CFU-S)

WBM cells from the femurs of 8-week-old C57Bl/6 mice were incubated *ex vivo* with (1 μM per 10^6 cells) dmPGE2, indomethacin, SC560, NS398 or ethanol control on ice for 2 h. Two independent bone marrow samples were treated (n = 5 per treatment, 2 replicates) for each

variable. Recipient mice were lethally irradiated with a split dose of 10 Gy. Sixty thousand unfractionated dmPGE2 or control-treated bone marrow cells were injected retro-orbitally into irradiated recipient mice. Spleens were dissected on day 8 or 12, weighed and fixed with Bouin's solution; haematopoietic colonies per spleen were counted. Cells (1×10^5 per recipient) were transplanted after treatment with the COX inhibitors. FACS-sorted $cKit^+Sca1^+Lineage^-$ bone marrow cells were treated as above and transplanted at a dose of 100 or 300 cells per recipient.

5-fluorouracil bone marrow injury

Mice were treated with 5-FU (150 mg kg^{-1}) as described[13]. SC560, NS398, dmPGE2 (1 mg kg^{-1}) or ethanol control were administered by intraperitoneal injection on days 1, 5, 9, 13 and 17 post injection. Peripheral blood was obtained on day 7 and 14, quantified and subjected to multilineage FACS analysis using antibodies (eBioscience) to B220/IgM (B-lymphoid), CD4/8 (T-lymphoid), Mac1/Gr1 (myeloid), Ter119/CD71 (erythroid) and cKit/Sca1 (stem/progenitor). Mice were killed on day 16, and bone marrow was isolated, quantified and analysed by FACS.

Limiting dilution competitive transplantation

WBM from CD45.1 C57Bl/6 mice was incubated with dmPGE2 or ethanol control *ex vivo*, as described. Treated test cells were independently transplanted into irradiated CD45.2 recipients ($n = 5$ per variable, 2 replicates) with untreated CD45.1/CD45.2 competitor at the following ratios: 15,000:200,000 (0.075:1), 50,000:200,000 (0.25:1), 200,000:200,000 (1:1) or 2,000,000:200,000 (10:1). Peripheral blood was obtained at 6, 12 and 24 weeks post transplantation, and white blood cells were FACS-analysed to determine test reconstitution for each series of treatment populations. Frequency of peripheral blood chimaerism >5% was used to calculate the number of repopulating cells using the L-Calc program (Stem Cell Technologies). For 12- and 24-week peripheral blood samples, multilineage reconstitution was measured by FACS analysis as above.

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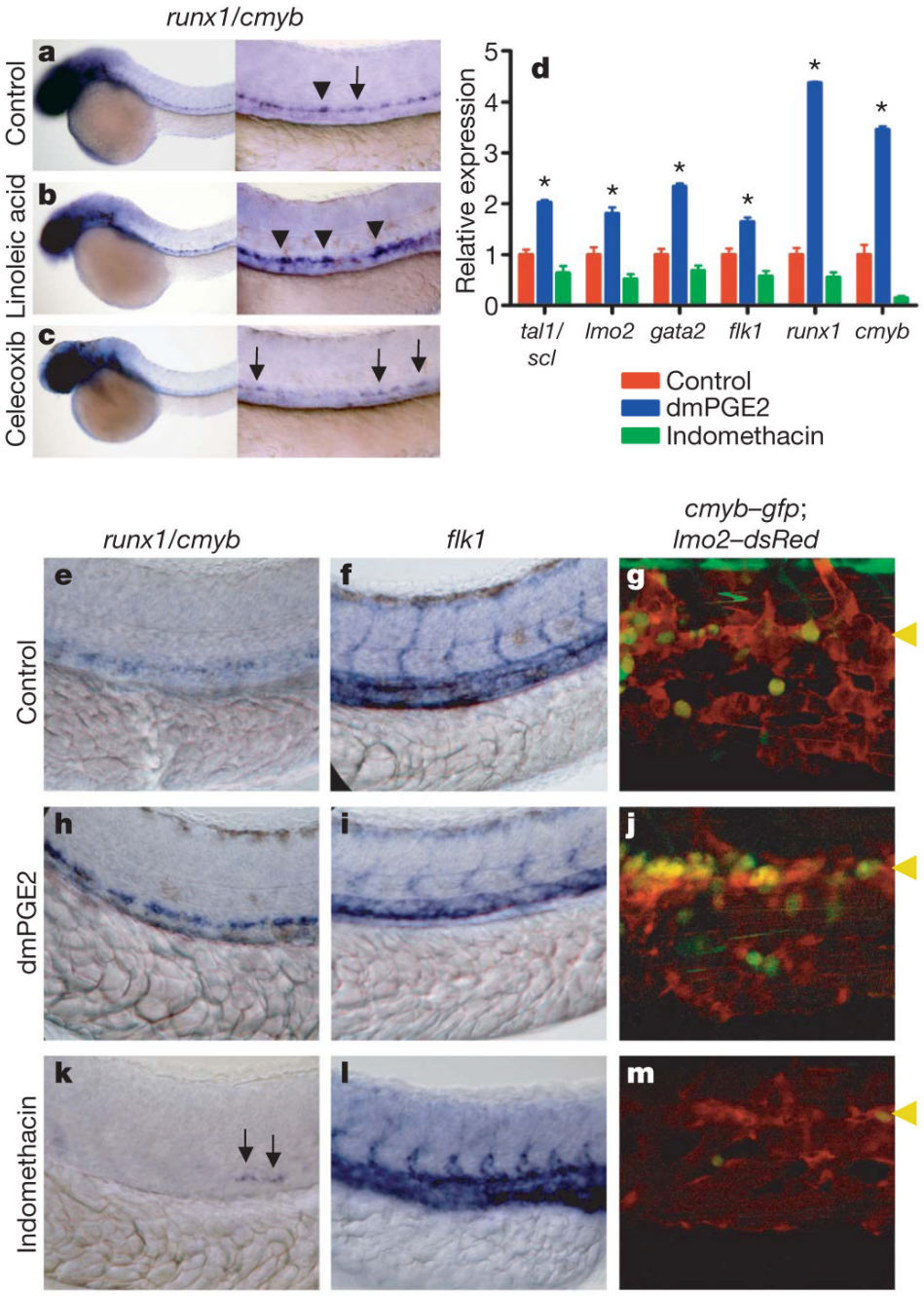

Figure 1. Prostaglandin agonists and antagonists alter *runx1/cmyb* expression without affecting vascular development

In situ hybridization for *runx1/cmyb* or *flk1* at 36 h.p.f. Photomicrographs were taken with Nomarski optics at 10× (**a-c**, left panels) and 40× (**a-c**, right panels, and **e-m**) magnification. **a-c**, Representative examples of chemicals in the prostaglandin pathway discovered in the screen are shown; 10 μM linoleic acid increases, and 20 μM celecoxib reduces HSC numbers. *runx1*$^{+}$/*myb*$^{+}$ HSCs are indicated: endothelial cells (arrow); haematopoietic clusters (arrowhead). **d**, Quantitative PCR profile of endothelial and HSC-specific gene expression following exposure to long-acting dmPGE2 (10 μM, blue) or the nonspecific Cox inhibitor indomethacin (10 μM, green) versus control (red). Both treatments resulted in statistically

significant differences compared with controls for each gene examined (ANOVA, $*P < 0.05$; mean, s.d. and *n* are listed in Supplementary Table 2). **e**-**m**, dmPGE2 and indomethacin exert opposing effects on *runx1/cmyb* expression by *in situ* hybridization (**e, h, k**); *flk1* is used to assess the effects on vascular development (**f, i, l**). Confocal microscopy images of *cmyb-gfp*; *lmo2-dsRed* bigenic fish exposed to dmPGE2 and indomethacin showing an increase and decrease in HSC (yellow) number along the ventral wall (yellow arrowhead) of the aorta, respectively (**g, j, m**). Quantitative analysis of 10 embryos in each treatment group revealed significant differences in HSC numbers (Supplementary Fig. 1i).

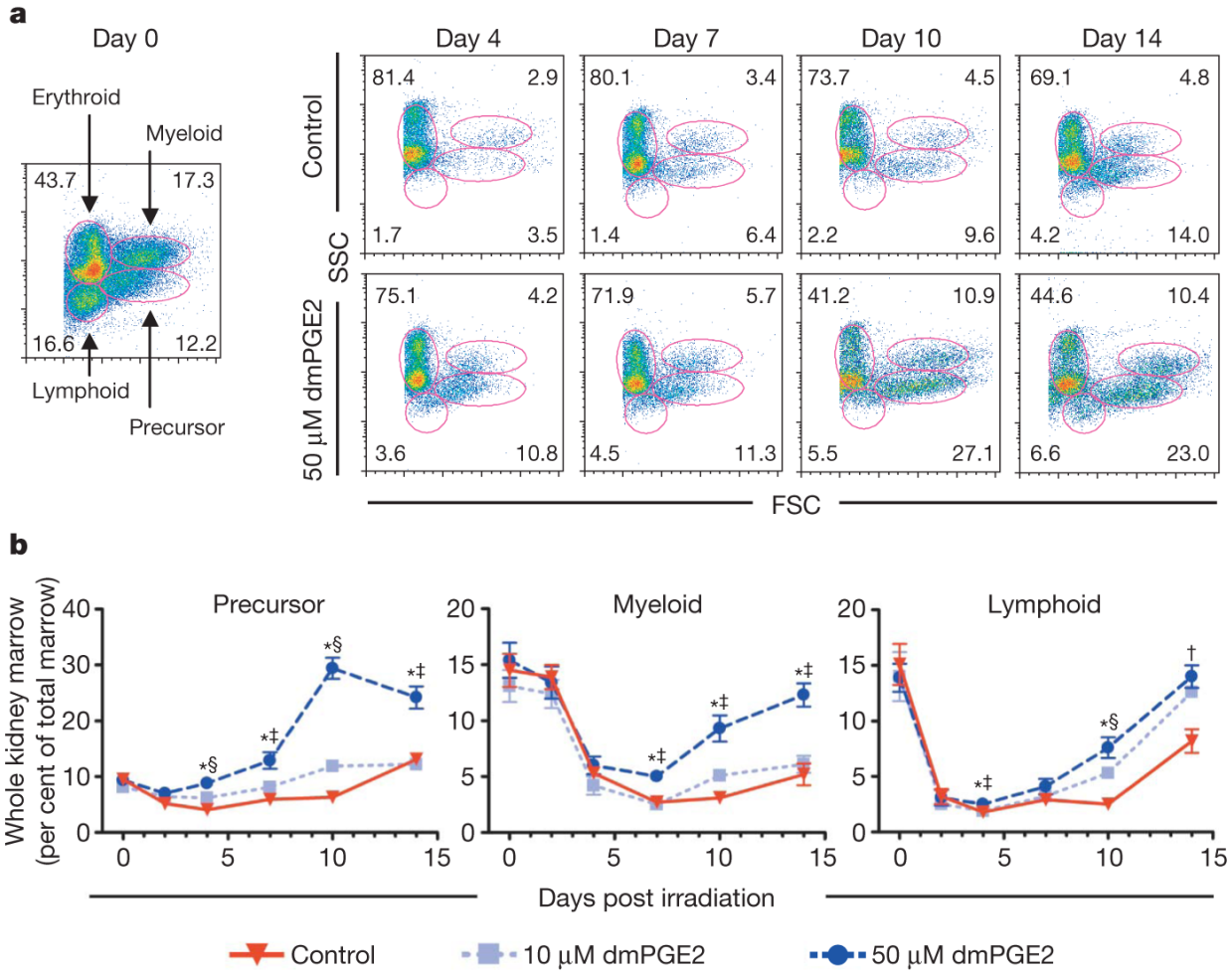

Figure 2. Treatment with dmPGE2 enhances haematopoietic recovery in sublethally irradiated adult zebrafish

Zebrafish whole kidney marrow irradiation recovery experiments were performed. **a**, Representative FSC/SSC FACS profiles of haematopoietic cell lineages in the kidney marrow on days 0, 4, 7, 10 and 14 of irradiation recovery in DMSO and dmPGE2-treated (50 µM) zebrafish. **b**, Kinetics of kidney marrow reconstitution of precursor, lymphoid and myeloid cells in control and dmPGE2-treated fish. Statistically significant differences: †, 50 µM versus control; ‡, 50 µM versus 10 µM, and 50 µM versus control; and §, all variables significant (ANOVA, *$P < 0.05$; mean, s.d. and *n* listed in Supplementary Table 3).

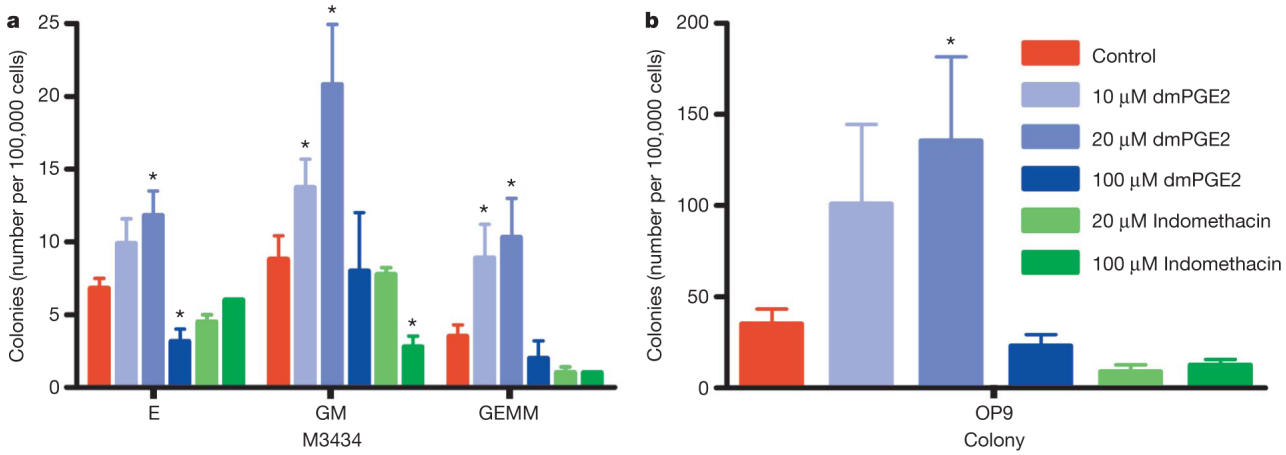

Figure 3. dmPGE2 modulates colony number and haematopoietic differentiation in mouse embryonic stem cells

M3434 and OP9 embryonic stem cell colony-forming assays were performed; counts are per 100,000 cells plated. An asterisk (*) indicates a statistically significant difference (two-tailed *t*-test; mean, s.d. and *n* listed in Supplementary Table 4). **a**, Effect of increasing doses of dmPGE2 and inhibition of cyclooxygenase activity by indomethacin on haematopoietic differentiation in methylcellulose; numbers of definitive erythroid (E), mixed granulocyte/monocyte (GM), and multi-potent (GEMM) progenitor colonies are shown (10 μM dmPGE2: GM, $P = 0.005$; GEMM, $P = 0.017$; 20 μM dmPGE2: E, $P = 0.04$; GM, $P = 0.007$; GEMM, $P = 0.016$; 100 μM indomethacin: GM, $P = 0.024$). **b**, Effect of dmPGE2 and indomethacin on OP9 haematopoietic colony number (20 μM dmPGE2, $P = 0.047$).

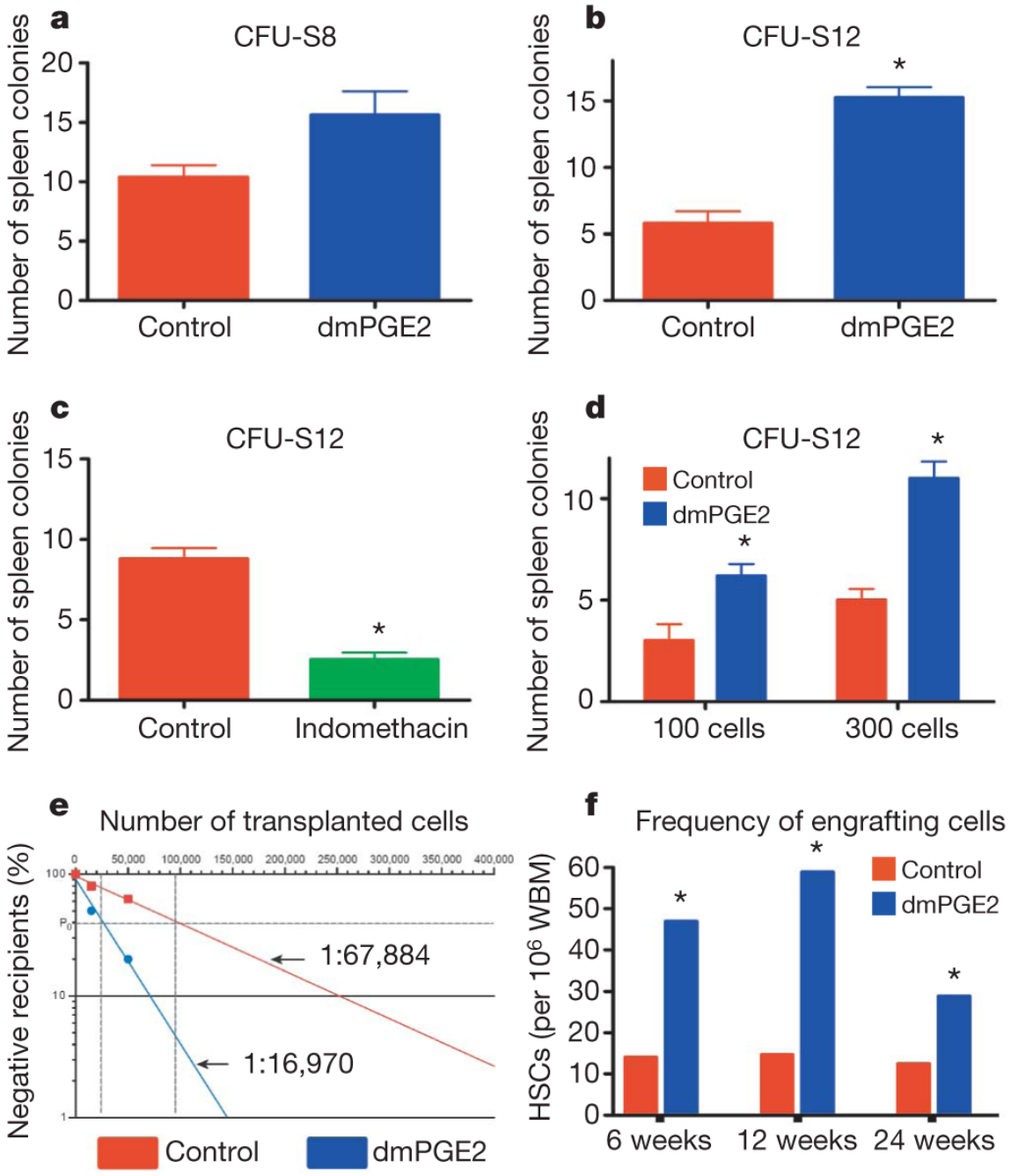

Figure 4. Exposure of murine bone marrow to dmPGE2 increases the number of CFU-S and repopulating HSCs

An asterisk (*) indicates a statistically significant difference. **a, b**, Effect of *ex vivo* treatment of WBM (2 h on ice) with ethanol control (red) or dmPGE2 (1 μM per 10^6 cells) on CFU-S8 and CFU-S12 (60,000 cells per recipient; CFU-S12: two-tailed *t*-test, control (mean/s.d./*n*) = 5.78/2.73/9, dmPGE2 = 15.22/2.39/9, $P < 0.0001$). **c**, Effect on CFU-S12 following *ex vivo* treatment with indomethacin (1 μM per 10^6 cells) (100,000 cells/recipient; two-tailed *t*-test, control (mean/s.d./*n*) = 8.8/2.10/10, indomethacin = 2.5/1.43/10, $P = 0.0001$). **d**, CFU-S12 evaluation after treatment of $cKit^+Sca1^+Lineage^-$ stem cells with dmPGE2 or ethanol control (two-tailed *t*-test, 100 cells: control (mean/s.d./*n*) = 3/1.63/4, dmPGE2 = 6.2/1.3/5, $P = 0.013$;

300 cells: control (mean/s.d./*n*) = 5/1.22/5, dmPGE2 = 11/1.87/5, $P = 0.0003$). **e, f**, Limiting dilution competitive repopulation assay. The number of negative recipients as determined by FACS analysis (**e**) in relation to the total number of cells transplanted for control or dmPGE2-treated cell samples is shown at 12 weeks. $P_0 = 67{,}884$ (control) and 16,970 (dmPGE2 treated). The frequency of engraftment (**f**) at 6, 12, and 24 weeks post transplantation in recipients of ethanol- versus dmPGE2-treated WBM calculated by Poisson statistics (ANOVA, $n = 10$ per variable; 6 wks, $P = 0.005$; 12 wks, $P = 0.002$; 24 wks, $P = 0.05$); the number of recipients surviving to analysis at each time point is shown in Supplementary Tables 7-9.