

Hepatocyte growth factor accelerates thrombopoiesis in transgenic mice

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Hepatocyte growth factor (HGF) is one of the potent growth factors for liver regeneration and has a strong effect on epithelial and nonepithelial cells. As one of the pleiotropic functions, HGF acts as a hematopoietic regulator in the proliferation and differentiation of hematopoietic progenitors. However, the effect of HGF on the thrombopoietic function remains unclear. The correlation between HGF and thrombopoiesis was investigated in transgenic (TG) mice overexpressing murine HGF controlled by the murine HGF by the metallothionein promoter. Furthermore, the mechanism of thrombocytosis induced by HGF *in vitro* was analyzed in hepatoma cell line HepG2. Both the platelet count and the serum thrombopoietin (TPO) concentration were significantly higher in TG than in the wild type (WT) control mice. In the liver and spleen, the expression of TPO mRNA in TG was higher than that in WT by real-time polymerase chain reaction. The expressions of transcriptional factor of TPO, GABP-alpha/beta were more increased in TG liver compared to WT. In an *in vitro* study, HGF induced TPO and GABP-alpha/beta expression and enhanced TPO promoter activity. Therefore, HGF induced thrombopoiesis accompanied with the overexpression of TPO through GABP stimulation.

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Hepatocyte growth factor (HGF) is a polypeptide originally characterized as a highly potent hepatocyte mitogen.^{1,2} Recent studies have revealed HGF to be a multifunctional cytokine which can elicit mitogenic, motogenic and morphogenic responses in a variety of cultured epithelial cells expressing the transmembrane tyrosine kinase receptor, c-Met.^{3,4}

HGF plays an essential role in hematopoiesis.^{5–8} HGF or c-met null mice showed defects in the liver and placental development and the migration of myogenic precursor cells into the limb bud.^{9–11} The erythrocyte count in embryonal blood has been reported to decrease in HGF null mice, although fetal hematopoiesis occurred in the impaired liver. This might be reflected in the reduction of the size of the liver and extensive cell death in HGF null mice.¹¹ On the other hand, the effect of HGF on thrombopoiesis and thrombopoietin (TPO) remains controversial.^{12–14}

TPO is the most potent factor promoting megakaryocyte growth and platelet production and is mainly synthesized in the liver.^{15,16} Several groups reported the cloning of the gene encoding TPO in 1994.^{15,17–19} TPO binds to its receptor,

c-Mpl, which is expressed on the megakaryocytes and platelets. The serum TPO levels are mainly regulated by the platelet count, because the platelets express a significant number of high-affinity c-Mpl receptors^{20–22} that can capture and degrade TPO.^{23–25}

We therefore investigated the correlation of HGF and thrombopoiesis. We thus found a new phenotype of HGF transgenic (TG) mouse thrombocytosis, although the number of white blood cells and erythrocytes did not change. We herein describe thrombocytosis in HGF TG mice and investigate the mechanism of thrombocytosis during the overexpression of HGF.

MATERIALS AND METHODS

Generation of HGF TG Mice and Control Mice

TG mouse, in which the expression of a murine HGF cDNA was driven by the metallothionein promoter and locus control regions, were generated on the inbred albino FVB/NCr genetic background (hereafter referred to as FVB) as previously described.²⁶ Briefly speaking, the transgene expressed a characteristic 2.4-kb RNA in virtually all adult tissues, at

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level between 3- and 50-fold higher than the major 6-kb endogenous HGF transcript. In addition, serum HGF level of TG was about four times higher than that of WT.²⁶ Six to 8-week-old male and female HGF TG mice and FVB wild type (WT) mice were used. WT and TG were placed at weaning (3 week of age) on a normal chow diet. No additional zinc was added because the transgene HGF induced by metallothionein promoter was sufficiently driven by the zinc in the usual diet. All animal studies were performed according to the guidelines for animal care and use as established by the Gunma University Graduate School of Medicine.

Peripheral Blood Cell Count and Histological Analysis of Bone Marrow and Liver

Peripheral blood was collected from axillary artery and blood cells were counted by auto-cell counter (SE-9000, Sysmex, Kobe, Japan). The femoral bone marrows of mouse were obtained and fixed in 10% formalin. The bone marrow samples were then stained with hematoxylin and eosin (HE) staining. We counted the number of megakaryocytes per high-power fields ($\times 400$). To examine the difference of the cell proliferation between WT and TG megakaryocytes, we performed PCNA staining^{27,28} on paraffin section of mice bone marrow using monoclonal mouse anti-PCNA antibody (PC-10; Dako Japan, Tokyo, Japan). The PCNA positive megakaryocytes were scored by counting 30 high-power light microscope fields ($\times 400$) for each group. The liver was stained with HE and then specimens were compared between WT and TG.

Measurement of Murine TPO

The plasma TPO concentrations in mice were measured by an enzyme linked immunosorbent assay.²⁹ Microtiter black plates (Sumilon, Osaka, Japan) were incubated with 5 $\mu\text{g/ml}$ rabbit anti-rmTPO IgG antibody at 4°C overnight. After washing, the bound murine TPO was detected using biotinylated chicken anti-mTPO IgG antibody (1 $\mu\text{g/ml}$), followed by alkaline phosphatase-labelled streptavidin and Lumigen PPD (Wako Chemical Co., Osaka, Japan), a chemiluminescent reagent, as the substrate. The chemiluminescent light emission was measured in a luminometer (Top Count; Packard, Meriden, CT, USA). The lower limit of sensitivity of the assay was approximately 50 pg/ml.

TPO Receptor Mpl Expression on the Platelet Surface

The peripheral blood was hemolysed with PharmLyse (BD Biosciences, San Jose, CA, USA), washed with the buffer (PBS containing 1 mmol/l of EDTA, 0.05% NaN_3 , and 0.3% BSA) and incubated with 10 $\mu\text{g/ml}$ of rabbit anti-murine c-Mpl polyclonal antibody (Amgen Inc., West Greenwich, RI, USA) plus 10 $\mu\text{g/ml}$ of FITC-labeled hamster anti-murine glycoprotein V monoclonal antibody (Seikagaku Corp., Tokyo) for 30 min at 4°C. Blood cells were washed and incubated with PE-labeled goat anti-rabbit immunoglobulin polyclonal antibody (Dako, Glostrup, Denmark) for 30 min at 4°C. The

cells were washed and analyzed on FACS Calibur (BD Bioscience, San Jose, CA USA). The expression of platelet surface c-Mpl was analyzed by measuring the fluorescence intensity of the cells in the glycoprotein V-positive fraction.

Cell Lines

The human hepatocarcinoma cell, HepG2 used in this study was originally obtained from the American Type Culture Collection (Manassas, VA 20108, USA). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco BRL, Grand Island, NY, USA) containing 10% fetal bovine serum (Equitech-Bio, Ingram, TX, USA), 50 U/ml penicillin, and 50 $\mu\text{g/ml}$ streptomycin (Gibco BRL). Cells were incubated at 37°C in a humidified chamber of 5% CO_2 .

Reagent

Recombinant Human HGF was purchased from R&D Systems Inc., Minneapolis, MN, USA. In order to confirm the effect of HGF signal, a specific mitogen-activated protein kinase/extracellular signal-related kinase (MEK) inhibitor PD98059 (Sigma-Aldrich Japan, K.K.Tokyo, Japan) was co-cultured with HGF.

Transient Transfection and Reporter Assay

To determine whether HGF induction of TPO gene expression is regulated at the transcriptional level, we performed promoter studies using the human TPO promoter. We used human TPO promoter-luciferase reporter plasmids with the 5'-flanking sequence of the TPO gene using pLUC-basic plasmid (Promega, Madison, WI, USA), constructed and kindly provided by Professor Kitajima.³⁰

The reporter plasmid (pLUC-Basic, pLUC-T58, pLUC-T88 and pLUC-T158) were transiently transfected into HepG2 cells with the control hpRL-tk vector (Promega) by FuGENE 6 Transfection Reagent (Roche Diagnostics, Indianapolis, IN, USA). At 3-h after transfection, 40 ng/ml of HGF was added to the dishes followed by incubation for 24 h. PD98059 was added 1 h before HGF treatment. The cells then were lysed and subjected to luciferase-based reporter assays using a dual luciferase assay system (Promega).

mRNA Analysis

Cells (2×10^5) were seeded in 35 mm-dish for 24 h then the medium were exchanged with serum-free DMEM and incubated over night. Furthermore these cells were incubated for 6 h with 40 ng/ml of the recombinant Human HGF, without or with 20 μM of PD98059 for 1 h before HGF stimulation.

Cells and mouse liver were trimmed and the total RNAs were extracted using ISOGEN (Nippon Gene, Tokyo) according to the protocol supplied by the manufacturer. Complementary DNA was synthesized from 5 μg of total RNA using SuperScript II reverse transcriptase and random hexamers (Invitrogen, Carlsbad, CA, USA).

Mouse *TPO*, *GABP-alpha/beta* which is the *Ets* family transcription factors were essential for the expression of the *TPO* in the liver,³⁰ and human *TPO*, *GABP-alpha/beta* mRNA were quantitated using real-time polymerase chain reaction (PCR) using TaqMan[®] fluorogenic probes (*TPO* and 18S ribosomal RNA; Applied Biosystems, Foster City, CA, USA) or SYBRgreen[®] detection (Mouse *GABP-alpha/beta* and human *TPO*, *GABP-alpha/beta*, Applied Biosystems). All primers except mouse *TPO* were designed using the Primer Express[™] design software (Applied Biosystems); sequence details are given in Table 1. Only mouse *TPO* primers and probe were obtained from Applied Biosystems assay on demand. PCR reactions and analyses were carried out using the ABI Prism 7700 Sequence Detector and software (Applied Biosystems) using mouse *actin* or human *glyceraldehyde-3-phosphate dehydrogenase (GAPDH)* as internal control. The relative copy numbers mRNA were calculated as recommended by the manufacturer.

Statistical Analysis

The values are the mean \pm s.d. of triplicate analysis from three independent experiments. Differences between the groups were analyzed by the Student's *t* test or one-way ANOVA. Comparisons between the groups are illustrated with box-plot graphics, where the dotted line within the box indicates the median value, and the box boundaries represent 50% of the values of non-outliers. The threshold for significance was set at $P < 0.05$.

RESULTS

Blood Cell Count

Table 2 shows both the blood white cell count and red cell count were not significantly different between WT and TG (2.96 ± 1.74 vs $3.25 \pm 1.24 \times 10^3$ cells/mm³, 7.63 ± 0.59 vs $7.29 \pm 0.70 \times 10^6$ cells/mm³, NS, respectively). The hemoglobin concentration and hematocrit were also similar between both mice. On the other hand, the platelet count was

higher in TG than WT (2219 ± 386 vs $967 \pm 145 \times 10^3$ /mm³, $P < 0.0001$).

Microscopic Findings of Bone Marrow and the Number of Megakaryocytes

To investigate *in vivo* hematopoiesis, bone marrow was decalcified and stained with HE (Figure 1a) and counted the number of megakaryocytes in high-power fields. The number of megakaryocytes of TG was significantly higher than that of WT (20 ± 2.5 vs 10.8 ± 1.55 per field, $P = 0.0003$) shown as Figure 1b.

PCNA Staining of Bone Marrow Cells

The number of PCNA positive megakaryocytes in TG bone marrow was clearly higher than that of WT (4.4 ± 0.55 vs 1.4 ± 0.55 per field, $P < 0.0001$) shown as Figure 1c and d.

Liver and Spleen Weight/Body Weight Ratio (Table 3) and Histological Analysis of the Liver

To rule out any basal characteristics in TG and WT, we measured the liver, spleen and body weight, and calculated the ratio between them. As a result, the liver weight was higher in TG than WT (2.00 ± 0.44 vs 1.23 ± 0.13 g, $P = 0.015$). However, the spleen weight was not different

Table 2 Hemogram of WT and TG

	WT	TG	P
WBC ($\times 10^3$ cells/mm ³)	2.96 ± 1.74	3.25 ± 1.24	0.77
RBC ($\times 10^6$ cells/mm ³)	7.63 ± 0.586	7.29 ± 0.700	0.43
Hb (g/dl)	11.7 ± 0.740	11.0 ± 1.21	0.31
Ht (%)	36.1 ± 2.10	35.3 ± 3.63	0.70
Plt ($\times 10^3$ /mm ³)	967 ± 145	2219 ± 386	< 0.0001

Table 1 Sequences of primer pairs used for amplification of mRNA by real-time PCR

mRNA	Accession number	Primer sense (5'-3')	Primer antisense (3'-5')
<i>Mouse</i>			
<i>actin</i>	NM_007393	GGCTCCTAGCACCATGAAGA	ACATCTGCTGGAAGGTGGAC
<i>GABP alpha</i>	NM_008065	GTACCAGATTATTATGCAAGACCG	TAAAGAAGATCGCCTACTGAGC
<i>GABP beta</i>	NM_207669	AGACCAACAAGAAGCCG	TAAAGTCCC GTTATCAAGCTGTAG
<i>Human</i>			
<i>GAPDH</i>	BC023632	GAAGGTGAAGGTCGGAGTC	GAAGATGGTGATGGGATTTTC
<i>TPO</i>	NM_199228	AGGTCCTGGACCAATC	TGGAAGAGGGAAGAGCG
<i>GABP alpha</i>	NP_002031	AAAGAGCGCCGAGGATTTGAG	CCAAGAAATGCAGTCTCGAG
<i>GABP beta</i>	NP_005245	CCCAGAGAGTCTGACACT	TCTGAAGAATTGGACAATGG

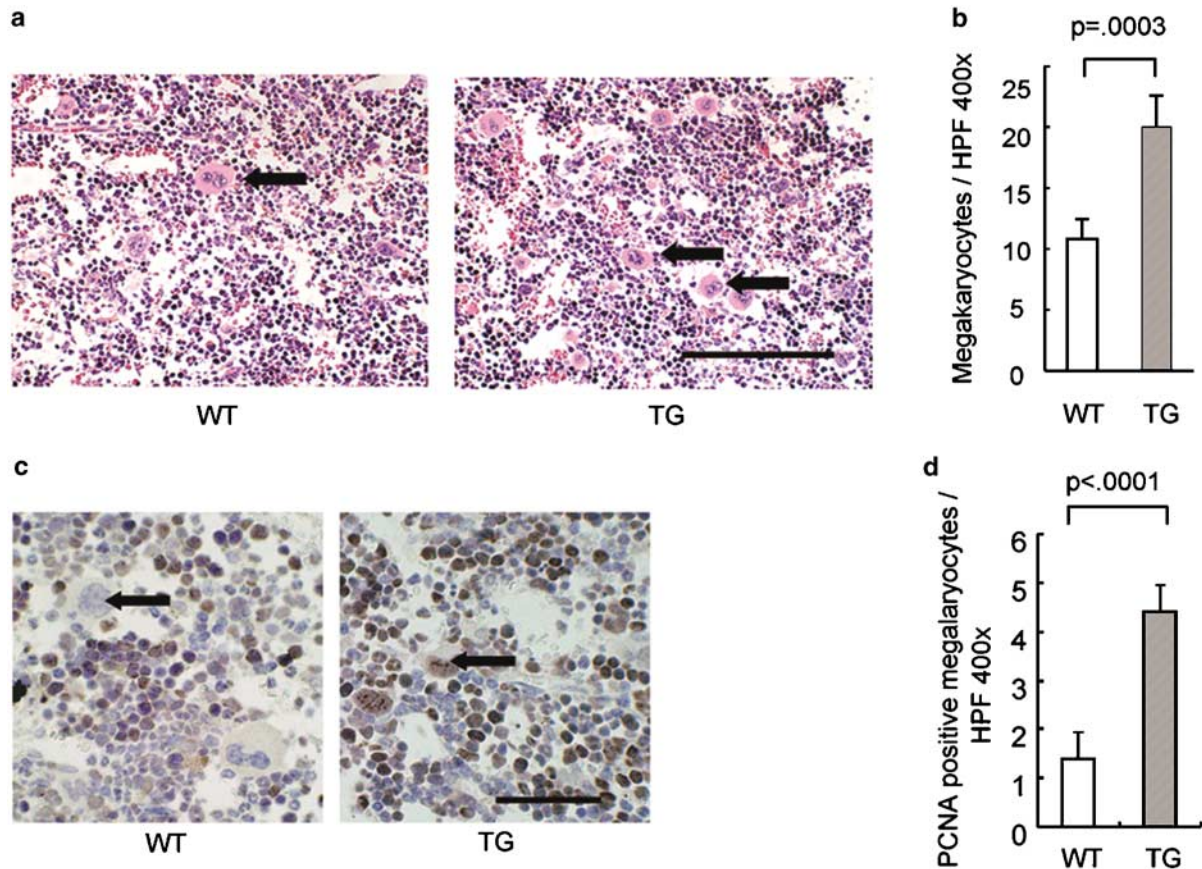


Figure 1 Histological findings of bone marrow. (a) Bone marrows of WT and TG were stained with hematoxylin and eosin. The megakaryocytes (arrows) showed a greater increase in TG bone marrow than in that of WT. $\times 400$, scale bars: $100\ \mu\text{m}$. (b) The number of megakaryocytes in TG bone marrow was statistically higher than that of WT. $P=0.0003$. (c) PCNA staining was clearly demonstrated more megakaryocytes were positive in TG than in WT. Bone marrow DNA synthesis in WT mice (left panel) and TG mice (right panel). Arrows show megakaryocytes. $\times 400$, scale bars: $50\ \mu\text{m}$. (d) The number of PCNA positive megakaryocytes in the WT or TG bone marrow. WT = white bars, TG = shaded bars. Error bars represent the standard deviation of triplicate experiments. Similar results were obtained in three independent experiments. $P<0.0001$.

Table 3 Liver and spleen weight

	WT	TG	<i>P</i>
Liver weight (g)	1.23 ± 0.13	2.00 ± 0.44	0.015
Spleen weight (mg)	108.8 ± 13.9	99.9 ± 24.0	0.21
Liver/body weight ratio (%)	5.06 ± 0.8	7.26 ± 1.1	0.0005
Spleen/body weight ratio (%)	0.386 ± 0.03	0.431 ± 0.06	0.11

between TG and WT (99.9 ± 24.0 vs 108.8 ± 13.9 mg, $P=0.21$). The liver/body weight ratio was higher in TG than WT (7.26 ± 1.1 vs 5.06 ± 0.8 %, $P=0.0005$). On the other hand, there was no difference between the TG and WT in spleen/body weight ratio (0.431 ± 0.06 vs 0.386 ± 0.03 %, $P=0.11$). At 8 weeks of age, the histological findings of TG liver did not grossly differ from that of WT liver. Moreover, no extramedullary hematopoiesis was demonstrated in both WT and TG liver (see Supplementary Information). The

weights of other organs (brain, femur muscle, heart, small intestine and kidney) were also not different between WT and TG (data not shown).

TPO Level in the Serum, c-Mpl Expression on Platelet and the Expression of TPO and GABPs in the Liver

The serum TPO concentration was higher in TG than WT (Figure 2a, 134 ± 49.3 vs 59.4 ± 16.5 pg/ml, $P=0.0012$). Furthermore, a positive correlation was recognized between the blood platelet count and the serum TPO level. Figure 2b shows that serum TPO level, which correlates with the liver weight, to be higher in TG than in WT (84.2 ± 40.4 vs 49.4 ± 17.5 pg/ml/g liver weight, $P=0.042$).

The mean value of c-Mpl expression of TG shifts to the left, in comparison to WT on flow cytometry. This result indicated that in TG the expression of c-Mpl is lower than that of WT (Figure 2c and d). Furthermore, the c-Mpl expression negatively correlated to the platelet count and serum TPO level (data not shown).

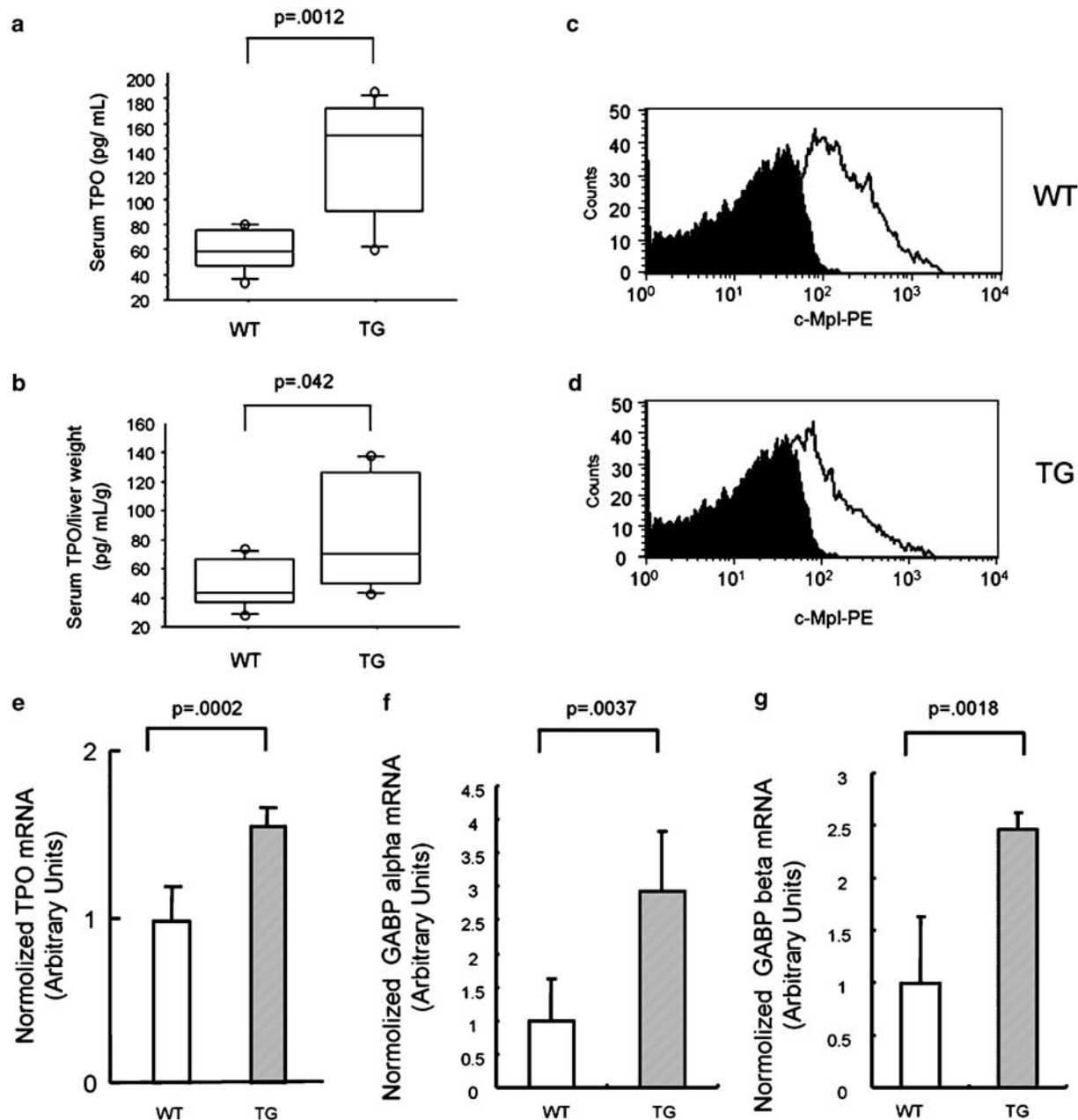


Figure 2 The serum TPO levels in WT and TG. (a) Serum TPO was compared between WT and TG. TG showed significantly higher TPO than WT. $P = 0.0012$. (b) Serum TPO per liver weight was compared between WT and TG because the liver is always larger in TG than WT. Comparisons between WT and TG are illustrated with box-plot graphics and the box boundaries represent 50% of the values of non-outliers. $P = 0.042$. Expression of c-Mpl on the platelets surface in WT (c) and TG mice (d). The expression of c-Mpl on the platelets analyzed with flow cytometry. The cells were stained with PE-labeled anti-c-Mpl (open area) or the isotype control antibody (filled area). The peak of Mpl expression in TG shifted left namely decreased in comparison to WT. The expression of TPO in the liver in WT and TG (e). TPOmRNAs were assessed by real-time PCR, and are expressed as the fold-induction relative to the WT liver. TPOmRNAs of TG were significantly higher than those of WT in the liver ($P = 0.0002$). Transcriptional factor GABP-alpha/beta expression in the liver. GABP-alpha (f) and -beta (g) mRNA were assessed by real-time PCR, and were expressed as the fold-induction relative to the WT liver. $P = 0.0037$, 0.0018 , respectively, WT mice = white bars, TG mice = shaded bars.

The TPO expressions in TG was significantly higher than those in WT in the liver (Figure 2e, $P = 0.0002$).

The Ets family transcription factors GABP-alpha and beta were more markedly upregulated in the TG liver than in WT (Figure 2f and g, 2.9- and 2.5-fold, respectively).

Expression of TPO, GABP-Alpha and -Beta Induced by HGF Stimulation

We investigated TPO induction by HGF *in vitro* using human hepatoma cell line HepG2. In addition, to examine the HGF/c-Met signaling, we used a MEK inhibitor, PD98059. TPO

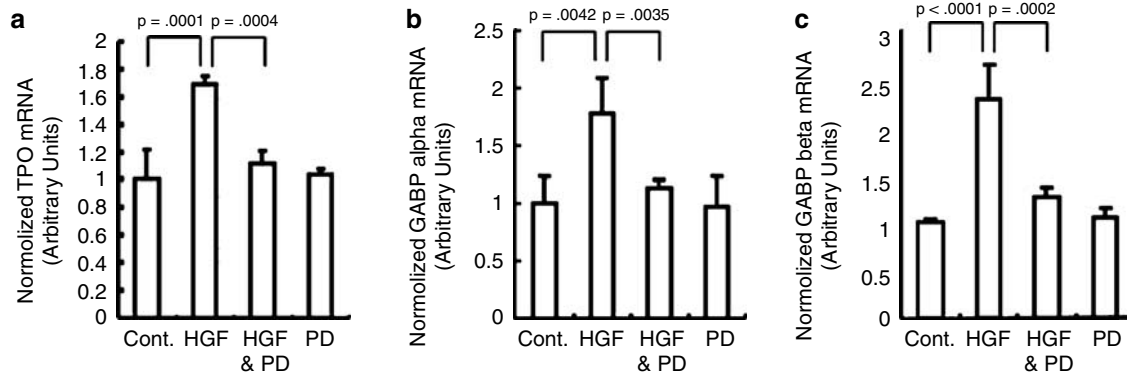


Figure 3 Effect of HGF and PD on the TPO and GABP mRNA expression in HepG2 cells. Real-time PCR analysis of mRNA of TPO (a), GABP-alpha (b) and GABP-beta (c). Lane 1, serum-free DMEM alone (Cont.); lane 2, HGF 40 ng/ml; lane 3, HGF 40 ng/ml and PD 20 μ M; lane 4, PD 20 μ M alone. Error bars represent the s.d. of triplicate experiments. Similar results were obtained in three independent experiments.

was induced 1.7-fold by HGF stimulation and a MEK inhibitor, PD98059 reduced the expression of TPO (Figure 3a). PD98059 alone had no effect on TPO.

Furthermore, Ets family transcription factor GABP-alpha (Figure 3b) and -beta (Figure 3c) were similarly induced by HGF stimulation (1.8- and 2.3-fold, respectively) and reduced by PD98059.

Effect of HGF and PD on TPO Promoter Activity in HepG2

To localize the region essential for human TPO gene expression, a series of 5'- to 3'-luciferase reporter plasmids were transiently expressed in HepG2 cells. As shown in Figure 4a, the plasmids containing 5' deletions of both lengths -158 and -88 promoted high level expression of luciferase activity in comparison to the background of pLUC-Basic. Moreover, HGF treatment strengthened these expressions. In contrast, a further deletion to -58 hardly promoted them. Furthermore, PD98059 inhibited TPO promoter induction by HGF, although not to the baseline. PD98059 alone had no effect on the TPO promoter activity (Figure 4b).

DISCUSSION

Thrombocytosis accompanied with TPO overexpression was clearly demonstrated in HGF TG in the present study.

In TG, HGF was always highly expressed in almost all internal organs including the liver and blood and the signal transduction of HGF through c-Met could be activated.^{26,31,32} On the other hand, TPO was reported to be primarily expressed in the liver and to a lower extent, in the kidneys, bone marrow and other organs.^{18,33,34} In a similar manner, we demonstrated the overexpression of TPO in the liver (Figure 2e). Hepatomegaly is one of the phenotypes of TG.³¹ Sakata *et al*³¹ had demonstrated that adult TG hepatocytes contained 4.7-fold greater proliferation index than WT by quantitating BrdUrd incorporation. Larger TG liver may induce a large amount of TPO in comparison to WT but

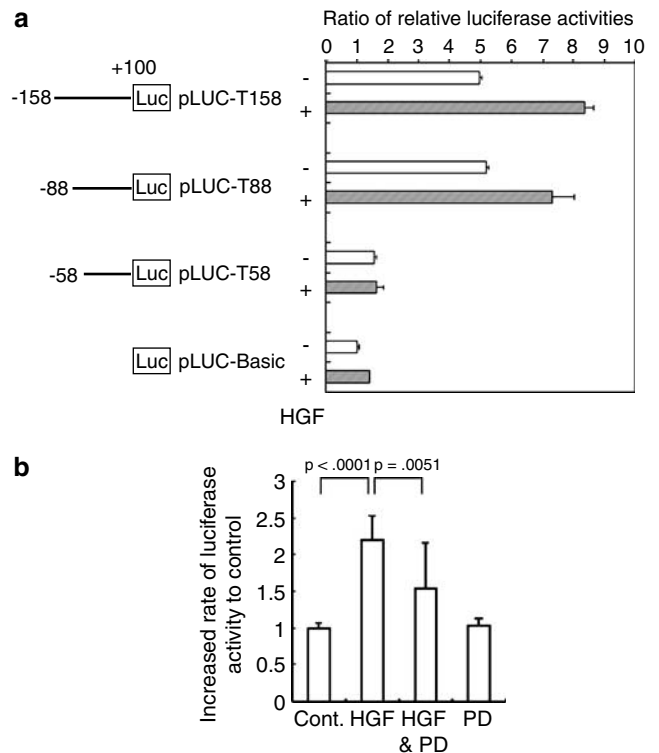


Figure 4 (a) Expression analysis of the various human TPO gene promoters constructs. Two micrograms of each reporter plasmid was transiently transfected into HepG2 cells with control vector to normalize the transfection efficiency. Luciferase assay was performed at 24 h after stimulation with or without 40 ng/ml HGF. Scheme of TPO promoter-luciferase fusion constructs (left panel). Relative luciferase activities using various plasmids are shown in the right panel. The relative luciferase activity of each construct was expressed as the fold-induction relative to non-treatment pLUC-Basic. Control group = white bars, HGF treatment group = shaded bars. (b) Effect of HGF and PD on the TPO promoter assay (pLUC-T158). Lane 1, serum-free DMEM alone (Cont.); lane 2, HGF 40 ng/ml; lane 3, HGF 40 ng/ml and PD 20 μ M; lane 4, PD 20 μ M alone. The relative luciferase activity of each construct was expressed as the fold-induction relative to control. Error bars represent the s.d. of triplicate experiments. Similar results were obtained in three independent experiments.

the TPO level was still higher in TG even when it was corrected by the weight of the liver (Figure 2b). As a result, the volume of the liver alone was thus not considered to be the cause of thrombopoiesis in TG.

Primary or essential thrombocytosis is a myeloproliferative disease caused by monoclonal or polyclonal abnormalities in hematopoietic cells. On the other hand, secondary or reactive thrombocytosis results from megakaryopoiesis and thrombopoiesis caused by infection, chronic inflammation, tissue damage (trauma, surgery, burns) or neoplasia.^{35,36} Neither monoclonal nor polyclonal abnormalities in hematopoietic cells were observed in TG. In addition, TG did not suffer from infection, inflammation or tissue damage. Although the mice could bear the liver tumor as the mice age,^{31,32} we used TG at a younger age before development of liver tumors. The cause of the thrombocytosis of our TG was none of the above.

Regarding the various functions of HGF, the thrombopoietic effect has yet to be fully elucidated. Banu *et al*³⁷ reported that HGF had no direct effect on megakaryocytes. The TPO induction by HGF *in vitro* has been controversial.^{12–14} In this study, we confirmed TPO induction by HGF with HepG2 cell line (Figure 3).

Furthermore, in our study *in vivo*, we demonstrated that platelet count, serum TPO level and TPO mRNA expression in liver were more increased in TG than in WT mice. Masunaga *et al*³⁸ and Yamashita *et al*¹⁴ also reported that HGF treatment caused thrombocytosis. Yamashita *et al*¹⁴ demonstrated that the administration of HGF to the cirrhotic rats stimulated TPO mRNA expression in the livers and resulted in significant increases of peripheral platelets and bone marrow megakaryocytes. However, the mechanism of TPO induction by HGF has yet to be clarified.

As one of the mechanisms of TPO mRNA induction by HGF, a transcription factor, Ets-1 is considered to be involved.^{39–41} As a direct pathway, HGF activates Ets-1 *in vitro*.^{39,40} Furthermore, Ets-1 is a downstream target of HGF acting through a RAS-REF-MEK-ERK pathway, could activate a signal transduction leading to the gene expression by HGF.⁴¹ The binding of Ets family transcription factors to the sequence 5'-ACTTCCG-3' in the human TPO promoter has been implicated in the expression of the TPO gene in the liver.³⁰ In TG liver, the expressions of Ets family, GABP-alpha and -beta, were significantly increased (Figure 2f and g). Kamura *et al*³⁰ revealed that GABP-alpha/beta were critical for the expression of the TPO gene in liver.

As shown in Figure 4a, positive regulatory elements were located from -88 to -58, and this region is essential for optimal transcription of the TPO gene. This result confirmed with the Ets motif recognized by E4TF1/GABP, which was regarded as the essential region for the high expression of the human TPO gene in the liver.³⁰ On the other hand, we demonstrated that HGF accelerated the expression of TPO, GABP-alpha/beta mRNA expression and the TPO promoter activity and additionally, MEK inhibitor, PD98059 inhibited

this expression and decreased promoter activity (Figures 3 and 4). These results implicated direct induction of TPO by HGF resulting thrombocytosis via the stimulation of Ets through activating MEK pathway.

HGF has been considered for the treatment of patients with liver cirrhosis,⁴² fulminant hepatic failure⁴³ and liver transplantation^{44,45} as well as arteriosclerosis.⁴⁶ As thrombocytopenia is often complicated in liver cirrhosis patients, HGF could thus be a choice for the treatment of thrombocytopenia as a therapeutic option for intractable diseases. However, we have previously shown that aberrant HGF expression induces several anomalies.^{26,31,32,47–50} The development of hepatic carcinoma makes use of HGF clinically not feasible.

In conclusion, HGF induced thrombocytosis with megakaryocytosis associated with the elevation of TPO in the TG liver and serum. As a part of the mechanism of this phenomenon, Ets family transcription factor GABP was induced by HGF in the liver. This is the reason why thrombocytosis was therefore accompanied with a high level of TPO induced by HGF. Taken all together, HGF induced thrombopoiesis accompanied with the overexpression of TPO through GABP stimulation. Therefore, HGF TG mice may thus be appropriate models of secondary thrombocytosis caused by a TPO overexpression induced by HGF.

Supplementary Information accompanies the paper on the Laboratory Investigation website (<http://www.laboratoryinvestigation.org>)

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