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Hepatocyte growth factor mobilizes non-bone marrow-derived circulating mesoangioblasts

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Aims The identification of factors that mobilize subsets of endogenous progenitor cells may provide new therapeutic tools to enhance the repair of ischaemic tissue. We previously identified circulating mesenchymal cells that co-express endothelial markers (so-called circulating mesoangioblasts, cMABs) in children undergoing heart surgery with cardiopulmonary bypass (CPB). However, the mechanisms by which these cells are mobilized and their origin is unclear.

Methods and results Circulating $CD73^+CD45^-KDR^+$ cMABs were analysed in adults undergoing heart surgery with ($n = 21$) or without CPB ($n = 8$). During surgery with CPB, cMABs are mobilized with a maximal response at the end of the operation. In contrast, off-pump heart surgery does not stimulate cMAB mobilization, indicating that the stress mediated by CPB induces the mobilization of cMAB. Circulating mesoangioblasts were enriched in blood obtained from the coronary sinus. Histologically, $CD73^+$ cells were detected around vessels in the heart, indicating that the heart is one of the niches of cMABs. Consistently, studies in gender mismatched bone marrow transplanted patients demonstrated that cMABs did not originate from the bone marrow. Cytokine profiling of serum samples revealed that hepatocyte growth factor (HGF) was profoundly increased at the time point of maximal mobilization of cMABs. Hepatocyte growth factor stimulated the migration of cMABs. Importantly, injection of recombinant HGF increased cMABs in rats.

Conclusions Hepatocyte growth factor induces mobilization of non-haematopoietic progenitor cells with a cardiac repair capacity. This newly identified function together with the known pleiotrophic effects of HGF makes HGF an attractive therapeutic option for the treatment of ischaemic heart disease.

Keywords Cell therapy • Mobilization • Cytokines

Introduction

Cell therapy is a promising option to improve the recovery of heart function after ischaemia or in patients with heart failure. Various different sources of stem and progenitor cells as well as pro-angiogenic cells have been shown to augment heart function after acute myocardial infarction (AMI).[1] The stem and progenitor cells tested include, but are not limited to haematopoietic progenitor cells (HPC), which are characterized by the expression of $CD34^+$ or $CD133^+$, as well as endothelial progenitor cells (EPCs) or pro-angiogenic cells, which can be cultured by several techniques leading to outgrowing endothelial cells or myeloid marker expressing cells that improve angiogenesis.[2–5] Mesenchymal stromal cells (MSC) isolated from the bone marrow or

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adipose tissue were also shown to increase the recovery after ischaemia.[6,7] Recently, we and others identified a subset of MSC that co-express endothelial markers in the embryonic aorta,[8] the adult mouse and human heart[9] and in the circulation.[10] On the basis of the absence of haematopoietic markers, the expression of typical mesenchymal markers and the expression of the endothelial marker VEGF receptor 2 (KDR), these cells were named mesoangioblasts (MABs).[11] Mesoangioblasts are distinct from early EPC and HPC since the haematopoietic markers CD45, CD34, and CD133 were not expressed.[10] Moreover, the lack of CD31 expression excludes that the isolated cells comprise outgrowing EPCs.[10] The detection of MABs in the circulation of children undergoing surgery implies that mechanisms must exist that induce the mobilization of these cells.

Trauma or coronary artery bypass surgery is well known to induce the mobilization of progenitor cells, but primarily haematopoietic or EPCs were detected.[12–14] Several hypoxia-modulated cytokines such as the vascular endothelial growth factor (VEGF) and stromal-derived factor 1 (SDF-1), as well as the colony stimulating factors G-CSF and GM-CSF, contribute to HPC and EPC mobilization.[15–19] Mesenchymal stromal cells were also reported to be mobilized into the circulating under physiological condition,[20] by severe stress conditions such as AMI,[21,22] by hypoxia[23] or by stimulation with colony stimulating factors such as GM-CSF.[24] However, the incidence of circulating MSC appears to be rather low[25] and some investigators failed to culture MSC after GM-CSF treatment.[26,27]

Here, we determined the clinical conditions favouring mobilization of circulating mesoangioblasts (cMABs) in children and adults and elucidated the underlying mechanism.

Methods

Cell isolation and culture from human peripheral blood

Blood samples were collected from children or adult patients undergoing open heart surgery with or without cardiopulmonary bypass. The baseline characteristics of children undergoing open heart surgery has been previously reported.[10] The patient characteristics of the adults is shown in *Table 1*. Written informed consent was obtained from each patient or patient's parent. The ethics review boards of the universities Giessen and Marburg, and Frankfurt approved the protocol, and the study was conducted in accordance with the Declaration of Helsinki.

Mononuclear cells (MNCs) were isolated by Ficoll density gradient centrifugation. Mononuclear cells were plated on fibronectin-coated dishes in EBM medium with supplemental growth factors and 20% foetal bovine serum. A similar protocol has been previously used for culturing early EPCs that were harvested at Day 4. However, CD45+ early EPC disappeared in culture at the third passage as confirmed by fluorescence activated cell sorting (FACS) analysis. In the present study, only outgrowing colonies were used that had been cultured for at least 4 weeks. Specifically, after 7 days, non-adherent cells were discarded and cells were cultured for additional 7 days with EBM medium. On Day 15, cells were detached by 0.25% Trypsin-EDTA (GIBCO) and were seeded at 5×10^4 cells/mL on fibronectin-coated dishes. When cells reached 80% confluence, cells were subsequently passaged at 5×10^4 cells/mL.

Flow cytometry

Following antibodies (Abs) were used for FACS: Phycoerythrin (PE)-conjugated anti-CD34, CD44, CD45, CD73 (BD Biosciences), CD144, KDR (R&D), biotinylated c-Met, allophycocyanin (APC)-conjugated streptavidin, and isotype-matched PE, FITC, or APC-conjugated mouse immunoglobulins. Circulating blood derived $CD34^+$ $CD45^+$ cells are detected as previously described[12] using the 8G12 clone (BD Biosciences). Samples were analysed by a flow cytometer, FACS Calibur cell sorter (BD Biosciences, San Jose, CA, USA).

Acute myocardial infarction model

Myocardial infarction was induced by permanent ligation of the left coronary artery in 10- to 12-week-old athymic NMRI nude mice (Harlan). Soon after ligation, 1×10^6 cells isolated from children or adults or PBS (all 50 μl) were injected intramuscularly into the border zone at three different sites as previously described.[10] After 2 and 4 weeks, echocardiographic analysis (Visualsonics; Vevo770) was performed. Additionally, cardiac catheterization was performed at 4 weeks for functional analysis by using 1.4 F micromanometer-tipped conductance catheter (Millar Instruments Inc.). Left ventricular pressure and its derivative were continuously monitored with a multiple recording system. All data were acquired under stable haemodynamic conditions.

Protein cytokine antibody array

Patient plasma was analysed by using a cytokine antibody array from RayBio Human Cytokine Antibody Array (RayBiotech, Inc., Norcross, GA, USA) according to the manufacturer's instructions. A semiquantitative analysis of the comparative intensity of the spots was performed with an image analysis program (TINA; version 2.09g).

Invasion assay of mesoangioblasts

Transwell membranes (8 μm; Corning Life Science) were coated with fibronectin (2.5 μg/mL; Roche, Mannheim, Germany) for 1 h. *Ex vivo*-expanded cMABs were incubated in the upper chamber at 37°C in 5% CO_2 for 4 h with or without hepatocyte growth factor (HGF; 500 ng/mL) in lower chamber. Migrated cells were counted after staining with DAPI.

Mobilization of mesoangioblasts

To confirm the mobilization of circulating MABs, 1 μg/kg recombinant human hepatic growth factor (rhHGF) or PBS was intravenously injected into Lewis rats. 2–8 mL of peripheral blood were collected from each rat and cell isolation and culture were performed as described above. In Vav-Cre-GFP mice, 1 μg/kg recombinant mouse HGF was injected.

Fluorescence *in situ* hybridization

Cells were fixed with 1% paraformaldehyd/PBS on ice for 10 min. The human X or Y probes were used for fluorescence *in situ* hybridization (FISH).[28] Probes were dehybridized for 4 min at 71°C before incubation of probes with samples (4 min at 80°C). Overnight hybridization at 37°C was followed by a stringent wash (2 × SSC with 50% formamide 2 times for 10 min, 2 × SSC for 5 min, NP-40/2 × SSC for 5 min, 2 × SSC for 5 min at 42°C). Nuclei were stained with DAPI (DAPI Mounting medium, Vector Laboratories). Images were obtained by confocal microscopy (Zeiss LSM510 system, Germany).

Table 1 Patient characteristics

	On-pump CABG $n = 11$	Off-pump CABG $n = 8$	Valve-replacement $n = 10$
Female sex, *n* (%)	0	1 (12.5%)	2 (20%)
Age (years)	61.1 ± 8.5	69.8 ± 9.6	61.8 ± 18.5
EF (%)	56.4 ± 13.8	52.5 ± 15.2	54.3 ± 11.1
No. of grafts	2.9 ± 0.7	2.4 ± 0.7	0
CPB (min)	87.6 ± 22.5	0	122.9 ± 44.4
Cross clamp (min)	49.6 ± 19.4	0	85.3 ± 32.5
Reop. for bleeding, *n* (%)	1 (9,1)	0	2 (20)
Neurolog. events, *n* (%)	1 (9,1)	0	0
Death *n*	0	1	0
Postop. MI, *n*	0	0	0
Hospital length of stay (days)	12.6 ± 6.2	9.5 ± 3.6	12.3 ± 4.1
Hypertension (%)	9 (81.8)	7 (87.5)	9 (90)
Hypercholesteraemia (%)	10 (90.9)	6 (75)	1 (10)
Diabetes (%)	5 (45.5)	2 (25)	2 (20)
Statins (%)	10 (90.9)	6 (75)	2 (20)
ACE inhibitor or AT-receptor blocker (%)	7 (63.6)	5 (62.5)	4 (40)

Statistics

Data are expressed as mean ± SEM. For comparison of two groups, two-tailed student's *t*-test (paired groups) or Mann–Whitney *U* test (unpaired groups) was used. For comparison of ≥3 groups, ANOVA with *post hoc* testing (Bonferroni) was used. Single regression analysis was performed to investigate the relation of MABs colony numbers and increase of plasma HGF. Statistical significance was assumed at a value of $P < 0.05$. All statistical analysis was performed with StatView (Version 5.0).

Additional methods are outlined in details in the Supplementary material online.

Results

Cardiopulmonary bypass during heart surgery stimulates mobilization of mesoangioblasts

Circulating mesoangioblasts were previously isolated from children undergoing heart surgery with cardiopulmonary bypass (CPB). Under these conditions, cMAB colonies were cultured in all 56 children that had been studied (for patient characteristics, see Koyanagi *et al.*[10]). In contrast, in children without heart surgery and CPB, cMABs were only obtained in 3 out of 10 children (30%). In adults, the difference was even more pronounced: cMABs were not detected in any individual without open heart surgery (0%), whereas circulating MABs were obtained in 18 out of 21 patients undergoing open heart surgery (85%). The cells isolated from adults were extensively analysed and showed similar characteristics compared with the cells isolated in children (*Figure 1A* and *B*, see Supplementary material online, *Figure S1*, for patients characteristic see *Table 1*). Consistent with the characteristics of children-derived cells,[10] the isolated cells from adults lack expression of the haematopoietic markers CD45 and CD34 discriminating the adult-derived cells from circulating EPC and HPC. Moreover, high expression of CD44 and CD73 distinguish the isolated cells from bone marrow-derived multipotent adult progenitor cells (*Figure 1A*, see Supplementary material online, *Figure S1*). Moreover, the expression of Oct4, Klf4, c-myc, as well as several cardiac transcription factors and typical MAB markers such as NG2 in adult-derived cMABs was similar compared with children-derived cMABs (*Figure 1B*/data not shown).

In order to determine whether cMABs isolated from children or adults also showed a similar capacity to augment cardiac function, we injected children- or adult-derived cMABs in nude mice after AMI. As shown in *Figure 1C* and *D*, children and adult-derived cMABs both augmented cardiac function to a similar extent. Taken together, these data indicate that open heart surgery with CPB mobilizes cMABs. Both children and adult-derived cMABs significantly enhanced the recovery of cardiac function after injection into an experimental model of acutely infarcted hearts.

In order to get more insights into the time-course of cMAB mobilization, we obtained serial blood samples from 10 patients before the operation, at the end of the operation prior to application of protamin used to block heparinization, and immediately (4–10 min) after the application of protamin and at Day 1 after the operation (*Figure 2A*). Whereas no colonies could be cultured from blood taken before the operation, the number of cMAB colonies cultured from blood samples peaked at the end of the operation prior to protamin application and returned to baseline at Day 1 (*Figure 2B*). In contrast, the kinetics of circulating $CD34^+$ cells was entirely different with increasing levels at Day 1 after the operation (*Figure 2C*). In order to determine whether the increase in cMABs is due to coronary bypass surgery, we additionally assessed cMAB mobilization in six patients undergoing open heart valve replacement with CPB. As shown in *Figure 2D*, the type of operation did not significantly affect the number of colonies cultured

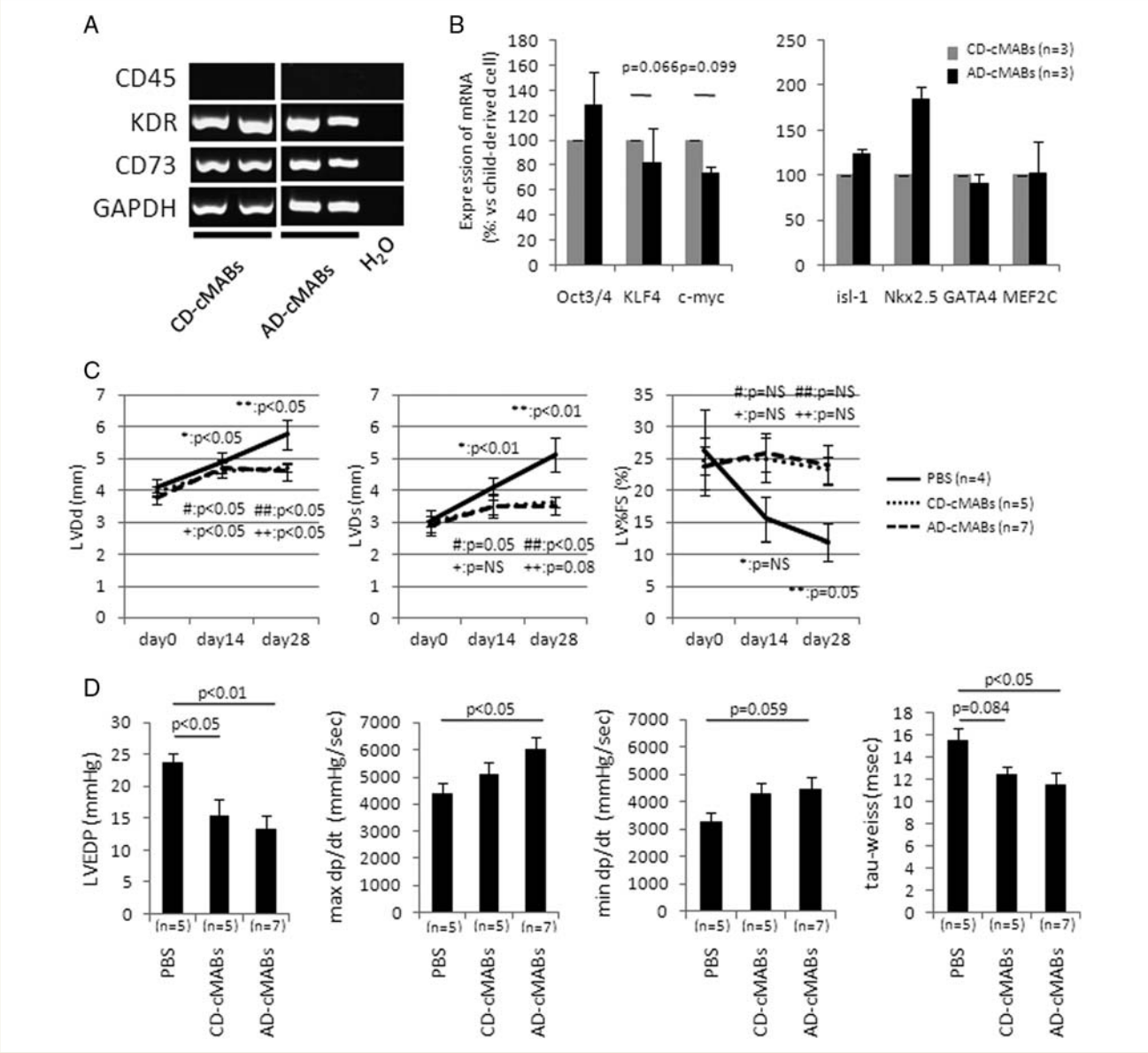

Figure 1 Characterization of children-derived and adult circulating mesoangioblasts. (*A*) Expression of markers for child (CD) and adult (AD) circulating mesoangioblast (cMABs). Representative RT–PCRs of haematopoietic (CD45), mesenchymal (CD73), and endothelial markers (KDR) are shown. H_2O was served as a negative control. (*B*) Microarray analysis of cDNA from child and adult circulating mesoangioblast. Expression of genes that induces pluripotency (Oct3/4, KLF4 and c-myc) and cardiac transcription factors (isl-1, Nkx2.5, GATA4, and MEF2C) were examined. $n = 3$ per group. For comparison of child-derived circulating mesoangioblast and ES cells see Koyanagi *et al.*[10] (*C*) Time course of echocardiographic parameters after induction of acute myocardial infarction (AMI) from Day 0 to Day 28 in PBS, child circulating mesoangioblast and adult circulating mesoangioblast treated mice. Change of left ventricular diastolic dimension (LVDd) and systolic dimension (LVDs) and fractional shortening (LVFS) are shown. One PBS-treated control mouse was excluded from echocardiography follow-up study, because of very poor echo image. * and ** represent *P*-values comparing Day 14 and Day 28 compared with the PBS group, respectively. # and ## represent *P*-values comparing Day 14 and Day 28 in adult-derived circulating mesoangioblast treated mice to Day 0, respectively; + and ++ represent *P*-values comparing Day 14 and Day 28 in children-derived circulating mesoangioblast treated mice compared with Day 0, respectively. (*E*) Pressure volume loops were measured at 28 days after induction of AMI. Left ventricular end-diastolic pressure (LVEDP), maximum (max d*p*/d*t*) and minimum (min d*p*/d*t*) d*p*/d*t* and tau-weiss are shown.

from blood samples. Finally, we investigated whether surgery alone without CBP is sufficient to induce the mobilization of cMABs. However, in patients undergoing coronary bypass surgery off-pump without CPB, cMABs colonies were rarely detected (*Figure 2D*), indicating that the stress imposed by CPB is responsible for the mobilization of cMABs.

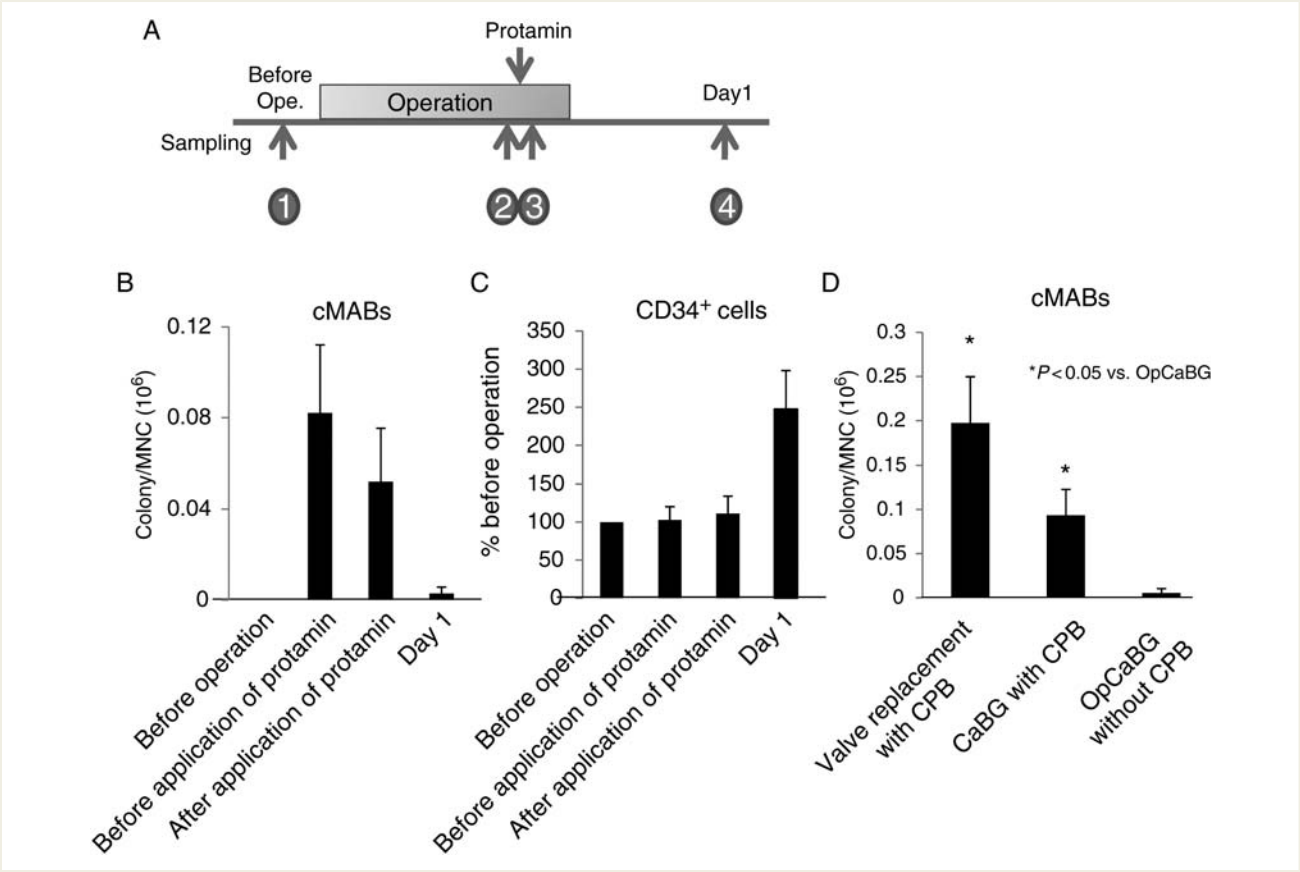

Figure 2 Mobilization of circulating mesoangioblasts in patients undergoing surgery. (*A*) Scheme illustrating the sampling of blood for culturing circulating mesoangioblasts. (*B* and *C*) Time-dependent mobilization of adult circulating mesoangioblast and CD34+ cells (*B*) circulating mesoangioblasts were isolated of blood samples obtained at the respective time points. The number of circulating mesoangioblast colonies was counted on Day 14. The number of colonies per 1×10^6 MNCs is shown. $n = 10$. $^*P < 0.05$ compared with before operation and Day 1. (*C*) $CD34^+$ $CD45^+$ cells were measured by fluorescence activated cell sorting in $n = 6$ patients with coronary artery bypass graft and cardiopulmonary bypass. (*D*) Circulating mesoangioblasts were isolated from blood samples obtained at the end of the operation before protamine treatment in patients with on-pump coronary artery bypass graft ($n = 11$), aortic or mitral valve replacement ($n = 6$), and off-pump coronary artery bypass graft ($n = 8$). Colonies were counted at Day 14.

Characterization of the origin of circulating mesoangioblasts

Having demonstrated that cMABs are mobilized during heart surgery with CPB in humans, we further aimed to elucidate the origin of cMABs. Bone marrow-derived HPC or EPCs are well established to be mobilized during trauma or injury.[14,29,30] Therefore, we analysed whether the cells giving rise to cMAB colonies originate from the bone marrow using blood from gender mismatched bone marrow transplanted children. As shown in *Figure 3A*, cultured cMAB colonies were exclusively derived from the recipient of the bone marrow transplant. Moreover, culturing cMAB colonies from Vav-Cre mice, which were crossed with stop-floxed GFP for lineage tracing of haematopoietic cells, revealed that the cultured cMAB colonies did not originate from the haematopoietic lineage (see Supplementary material online, *Figure S2*). These results indicate that cMABs do not originate from haematopoietic bone marrow-derived cells, in line with the lack of expression of haematopoietic markers.

Since previous studies demonstrated that MABs can be isolated from the human heart,[9,31] and $CD73^+$ cells can be detected around vessels in sections of human hearts (see Supplementary material online, *Figure S3*), we hypothesized that MABs may be mobilized from the heart itself. Therefore, we compared measurements of cMABs in blood samples obtained from the vena cava with blood samples obtained from the coronary sinus that drains the blood from the heart. Importantly, the number of cMAB colonies was significantly higher in the blood obtained from the coronary sinus (*Figure 3B*) supporting the concept that cMABs might be mobilized from the heart.

Hepatocyte growth factor mobilizes circulating mesoangioblasts

Cardiopulmonary bypass is well known to activate inflammatory cells and induce various cytokines.[32–35] Therefore, we profiled the levels of circulating cytokines before and at the end of the operation prior to protamine administration, at the time point

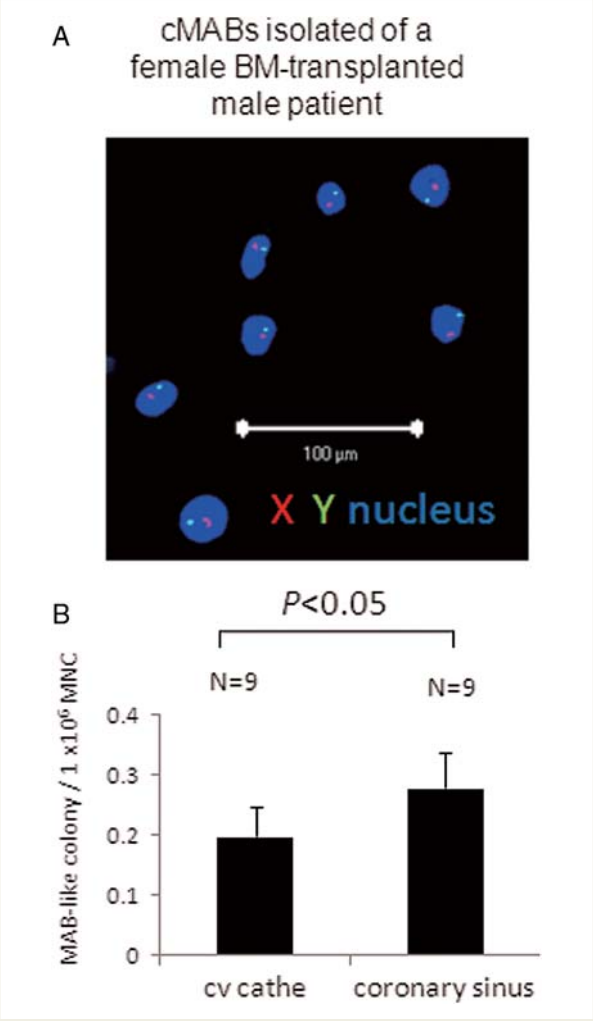

Figure 3 Circulating mesoangioblasts are non-bone marrow-derived. (*A*) Representative fluorescence *in situ* hybridization (FISH) staining of X and Y chromosome of expanded circulating mesoangioblast from gender mismatched bone marrow transplanted patients (recipient: male, donor: female). A representative example is shown. (*B*) Number of circulating mesoangioblast colonies cultured from blood samples obtained from central venous blood (CV catheter) and coronary sinus at the end of the operation. $n = 9$ patients.

were maximal levels of cMABs were obtained. As shown in *Figure 4A–C*, in both children and adults, the cytokines interleukin-6 and HGF were elevated at the time point of maximal mobilization of cMABs. The elevation of HGF plasma levels was confirmed by quantitative measurement of HGF by ELISA and the levels significantly correlated with the number of cMAB colonies (see Supplementary online, *Figure S4*/data not shown). Therefore, we further elucidated a potential biological effect of HGF on cMABs. First, we measured the expression of the HGF-receptor and demonstrated that cMABs abundantly express the HGF-receptor c-met (*Figure 5A*). Second, we determined whether cMABs respond to recombinant HGF. Indeed, HGF induced the invasion of cMABs *in vitro* (*Figure 5B*) indicating that cMABs respond to HGF. Taken together, HGF might indeed be a candidate to mobilize cMABs.

Therefore, we finally determined whether recombinant HGF is sufficient to stimulate the mobilization of cMABs *in vivo*. Injection of HGF in rats increased the number of colonies that express the typical markers of cMABs (*Figure 6A* and *B*). In contrast, the number of circulating lin-Sca-1 + c-kit + haematopoietic cells were not increased (data not shown).

Discussion

The data of the present study demonstrate that CPB elicits a stress response that induces the mobilization of a subset of mesenchymal progenitor cells, namely MABs. Mobilization of cMABs was specifically detected in patients undergoing heart surgery with CPB, whereas patients undergoing off-pump coronary artery bypass surgery did not show an increase in cMABs. Additionally, we demonstrate that mobilization occurred independently of the type of operation in patients with coronary artery bypass surgery or valve replacement with CPB. Maximal mobilization of cMABs was observed already at the end of the operation (mean CPB time 104.4 ± 38.3 min) and the kinetics was different compared with HPC that were mobilized at Day 1 and during the subsequent days after the operation. The time-dependent increase in HPC mobilization is in accordance with previous studies showing that different subsets of haematopoietic cells are increased within 6 h up to 7 days after coronary bypass surgery, trauma, or AMI (Scheubel *et al.*[36]; for review see Brunner *et al.*[37]). To identify a putative mechanism underlying the mobilization of MABs by CPB, we profiled the cytokines at the time point of maximal cMAB mobilization. Of note, only HGF was significantly up-regulated in children and adults undergoing surgery and CPB, whereas other cytokines that are known to be involved in stem/progenitor cell mobilization and migration such as G-CSF or SDF-1 were not elevated at the time point studied. This rather specific up-regulation of only few cytokines is in contrast to the demonstration of multiple dysregulated cytokines in this patient cohort.[32] However, most of the published studies showed that the profound augmentation of cytokines is maximal at later time points within 4–24 h after the end of the operation.[36,38] Thus, the early time point at which blood samples were obtained in the present study (prior to protamine addition) may be responsible for the limited effects on the cytokine levels. Hepatocyte growth factor levels correlated with the colony numbers of the mobilized MABs suggesting that this cytokine might mediate the mobilization in the patients. Indeed, we demonstrate that HGF induced the migration of *ex vivo* cultured cMABs and that HGF mobilized cMABs in a rat model indicating that HGF might play a crucial role for the mobilization of cMABs occurring during CPB surgery. However, we cannot exclude that CPB additionally induces other factors or modulates vascular permeability, thereby, facilitating mobilization of vessel-associated progenitor cells. Hepatocyte growth factor was previously shown to enhance SDF-1-mediated directional migration of CD34 + progenitors,[39] to stimulate migration of bone marrow MNCs *in vitro*,[40] and HGF is known to promote survival of progenitor cells.[41] However, this is the first study to our knowledge that

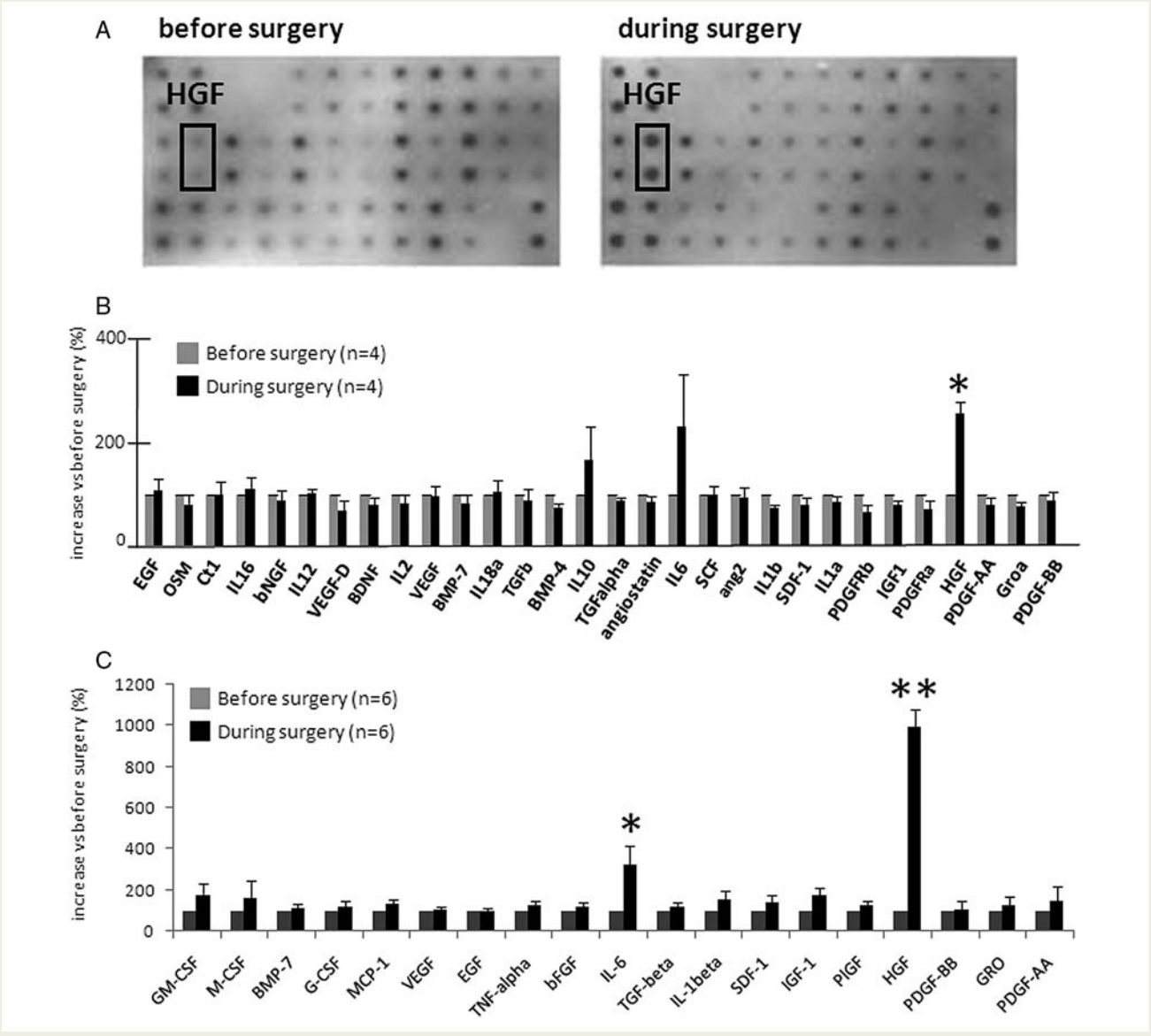

Figure 4 Cytokine profiling. (*A*) Representative pictures of the cytokine antibody array analysis. Blood samples were obtained before surgery and at the end of the operation. The blue squares indicate hepatocyte growth factor (HGF). (*B* and *C*) Quantification of the cytokine array analysis in children ($n = 4$) (*B*) and adults ($n = 6$) (*C*). The % increase compared with levels before surgery is shown. Abbreviations are as follows: OSM, oncostatin M; Ct1, cardiotropin-1; bNGF, beta-Nerve Growth Factor; BDNF, brain-derived neurotrophic factor. *: $P < 0.05$ vs. before operation, **: $P < 0.01$ vs. before operation.

demonstrates that HGF can affect mobilization of tissue-derived MABs. Hepatocyte growth factor was already used in several clinical trials for neovascularization improvement, either as recombinant protein or as plasmid.[42,43] On the basis of the demonstration that HGF application was not associated with adverse effects in these Phase-I/II studies, one may consider using HGF to mobilize cMABs for therapeutic purposes.

Finally, the present study provides first evidence that cMABs are tissue-derived. This is supported by demonstrating that cMABs do not originate from the bone marrow in gender mismatched bone marrow transplanted patients. Moreover, lineage tracing of haematopoietic cells by using the Vav-Cre reporter line[44] confirmed that cMABs do not originate from the haematopoietic lineage. Immunostainings showed that $CD73^+$ cells can be detected in the human heart particularly around vessels. Since CD73 expression characterizes cMABs and FACS sorting of circulating $CD73^+$ cells enriches cMABs,[10] these data would suggest that cMABs might originate from vessel-associated cells in the heart or other tissues. However, a limitation of the present studies is that this hypothesis cannot be finally proven since this point cannot be addressed in the clinical setting. Moreover, the absence of specific markers of MABs that could be used for lineage tracing precludes such a crucial experiment in the experimental model. However, several lines of evidence support this idea: First, MABs have

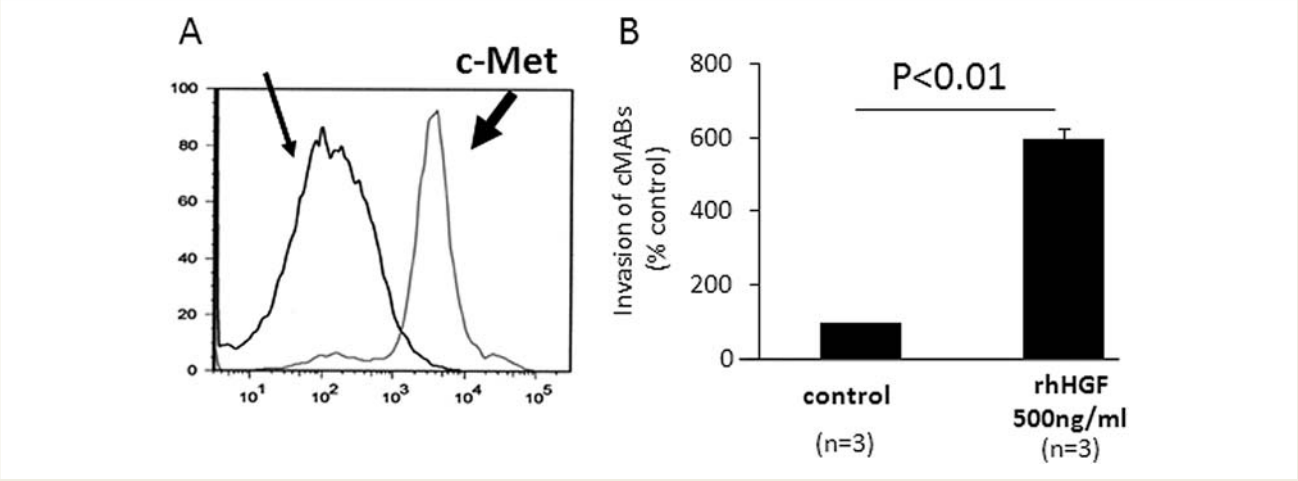

Figure 5 Hepatocyte growth factor activates circulating mesoangioblasts. (*A*) Expression of the hepatocyte growth factor receptor c-Met was determined in cultured circulating mesoangioblast by fluorescence activated cell sorting. (*B*) Invasion was stimulated with or without hepatocyte growth factor (500 ng/mL) and migrated cells were counted after 4 h. $n \geq 3$.

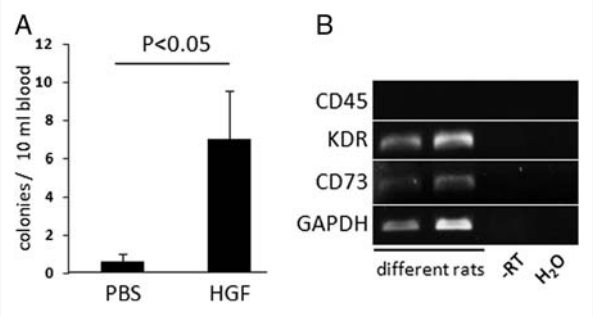

Figure 6 Hepatocyte growth factor mobilizes circulating mesoangioblasts. (*A*) HGF (1 μg/kg; $n = 7$) or PBS ($n = 4$) was injected intravenously in rats and peripheral blood was obtained 24 h after injection. Circulating mesoangioblasts were cultured and the number of circulating mesoangioblast colonies per 10 mL peripheral blood in each rat group is shown. (*B*) Surface marker expression of rat circulating mesoangioblast isolated from blood obtained from two individual hepatocyte growth factor injected rats. Representative RT–PCR of haematopoietic (CD45), mesenchymal (CD73), and endothelial markers (KDR) are shown. –RT and H_2O were served as negative controls.

originally been identified in the aorta during embryonic development demonstrating that these cells are indeed vessel-associated progenitor cells.[8] Second, cMABs are enriched in the coronary sinus that drains the blood of the heart compared with the levels in the systemic circulation. Finally, recent reports suggest that the HGF/c-met axis controls cardiac progenitor cells.[45,46] Since circulating cMABs isolated in the present study and previously characterized heart-derived MABs[9] express cardiac transcription factors, one may speculate that these cells might have been adapted to the cardiac environment. Particularly, heart-derived MABs but also cMABs were shown to differentiate to cardiomyocytes *in vitro* and *in vivo*. Although we demonstrated that transduction of cMABs with Sox2 further enhances the cardiac repair capacity, unmodified cMABs also were capable to express cardiac marker genes upon stimulation.[10] The functional benefit of cMABs isolated from children and adults is further supported by the demonstration of improved cardiac function in mice with AMI.

In summary, our data provide first evidence that tissue-derived MABs can be mobilized upon stress and particularly by the cytokine HGF. The mobilization of mesenchymal progenitor cells together with its additional pleitrophic beneficial effects makes HGF attractive for improving the recovery after ischaemia and tissue injury.

Limitations of the study

Although our data suggest that HGF mobilizes non-haematopoietic cells, the causal evidence that this indeed occurs in humans is not provided. Moreover, in the absence of lineage tracing experiments, it remains to be determined whether the cMABs studied are indeed derived from vessels in the heart or whether the cells may originate from other sources.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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Conflict of interest: none declared.

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CARDIOVASCULAR FLASHLIGHT

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Percutaneous double valve intervention

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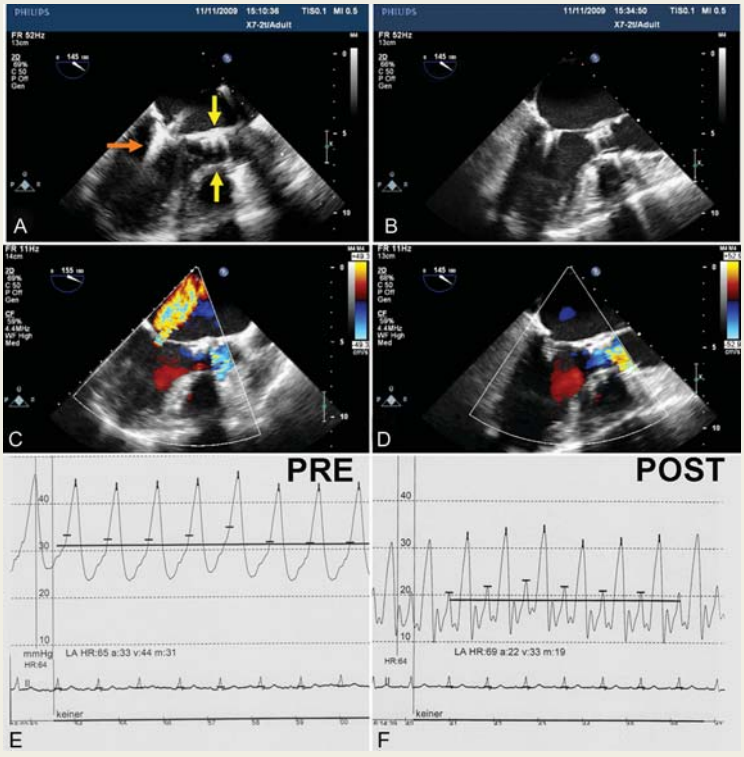
An 81-year-old woman was referred for NSTEMI complicated by cardiogenic shock. Urgent invasive assessment revealed a subtotal left anterior descending artery (LAD) stenosis, severe aortic stenosis (mean gradient 60 mmHg, aortic valve area 0.5 cm^2), decreased left ventricular (LV) function [left ventricular ejection fraction (LVEF) 49%], grade 3+ mitral regurgitation (MR), and severe pulmonary hypertension. In view of her critical situation, acute LAD–percutaneous coronary intervention and aortic valvuloplasty were performed with subsequent marked clinical improvement. Yet, she remained in NYHA class III. Given her critical condition, the likelihood of double valve operation and high predicted 30-day mortality (log EuroSCORE 74%, STS 30%), there was interdisciplinary consensus for catheter-based strategy, primarily treating the aortic stenosis possibly improving functional MR. Three weeks after the acute event, she underwent trans-femoral aortic valve implantation. However, despite an excellent technical result, LVEF and MR did not improve and she required 3 weeks ICU with intravenous catecholamines and diuretics administration. As severe MR and severe pulmonary hypertension persisted, we opted for percutaneous mitral valve repair (*Figure*). One MitraClip® was successfully implanted between segment A2 and P2 with an MR reduction to grade 1+ and a drop in mean left atrial pressure from 31 to 19 mmHg.



One week after percutaneous MR repair, she was discharged in stable conditions. Eight months after, the patient has remained in NYHA class I, whereas LV function remained stable (LVEF 55%).

At the best of our knowledge, this is the first report of a double cardiac valve intervention, performed solely by trans-femoral access. The presented case confirms improvement in trans-catheter valve interventions enabling the treatment of high-risk patients not suitable for open-heart surgery.

Figure. Echocardiography during MitraClip implantation. *Panel A*: open clip in mitral position (red arrow). Edwards 23 mm prosthesis in aortic position (yellow arrows). *Panel B*: closed MitraClip after implantation in definitive mitral position. The middle panels show the Color duplex examination of the mitral valve before (*Panel C*) and after (*Panel D*) successful implantation of one MitraClip. The bottom panels show the haemodynamic curves in the left atrium before (*Panel E*) and after MitraClip implantation (*Panel F*).

Supplementary material

Supplementary material is available at *European Heart Journal* online.